

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
1 May 2003 (01.05.2003)

PCT

(10) International Publication Number
WO 03/034980 A2

(51) International Patent Classification⁷: **A61K**
(21) International Application Number: PCT/US01/43089
(22) International Filing Date:
14 November 2001 (14.11.2001)
(25) Filing Language: English
(26) Publication Language: English
(30) Priority Data:
60/274,622 14 November 2000 (14.11.2000) US
(71) Applicant (for all designated States except US): **NEW RIVER PHARMACEUTICALS INC.** [US/US]; The Governor Tyler, 1902 Downey Street, Radford, VA 24060 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

(71) Applicant and
(72) Inventor: **PICARIELLO, Thomas** [US/US]; 203 Murphy Street, N.E., Blacksburg, VA 24060 (US).
(74) Agents: **SCHULMAN, Robert, M.** et al.; Intellectual Property Department, Hunton & Williams, 1900 K Street, N.W., Suite 1200, Washington, DC 20006-1109 (US).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 03/034980 A2

(54) Title: A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ABACAVIR SULFATE AND METHODS OF MAKING AND USING SAME

(57) Abstract: A composition comprising a polypeptide and benztropine mesylate covalently attached to the polypeptide. Also provided is a method for delivery of benztropine mesylate to a patient comprising administering to the patient a composition comprising a polypeptide and benztropine mesylate covalently attached to the polypeptide. Also provided is a method for protecting benztropine mesylate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of benztropine mesylate from a composition comprising covalently attaching it to the polypeptide.

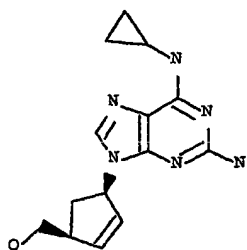
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ABACAVIR SULFATE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to abacavir sulfate, as well as methods for protecting and administering abacavir sulfate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Abacavir sulfate is a known pharmaceutical agent – a carbocyclic 2'-
15 deoxyguanosine nucleoside analogue that is a reverse transcriptase inhibitor used in the treatment of HIV. Its chemical name is (1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol. Its structure is as follows:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

CW001P

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW001P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

CW001P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent (abacavir sulfate) to a polymer of peptides or amino acids. The invention is distinguished from the above mentioned technologies by virtue of covalently attaching abacavir sulfate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
- 15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising abacavir microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and abacavir sulfate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,
- 25 (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

abacavir sulfate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting abacavir sulfate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering abacavir sulfate to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, abacavir sulfate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, abacavir sulfate is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and abacavir sulfate is released from the composition by dissolution of the microencapsulating agent. In another preferred

CW001P

embodiment, abacavir sulfate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, abacavir sulfate is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching abacavir sulfate to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, abacavir sulfate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize abacavir sulfate and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of abacavir sulfate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Abacavir sulfate is the subject of U.S. Patent Numbers 5,034,394 and 5,089,500, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises abacavir sulfate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW001P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW001P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW001P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW001P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW001P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, abacavir sulfate is covalently attached to the polypeptide via its alcohol group or, alternatively, its amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW001P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-abacavir sulfate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW001P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW001P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW001P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 abacavir sulfate covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein abacavir sulfate is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW001P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein abacavir sulfate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing abacavir sulfate from said composition in a pH-dependent manner.

15 19. A method for protecting abacavir sulfate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of abacavir sulfate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching abacavir sulfate to said polypeptide.

20 21. A method for delivering abacavir sulfate to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
abacavir sulfate covalently attached to said polypeptide.

25 22. The method of claim 21 wherein abacavir sulfate is released from said composition by an enzyme-catalyzed release.

CW001P

23. The method of claim 21 wherein abacavir sulfate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

A composition comprising a polypeptide and abacavir sulfate covalently attached to the polypeptide. Also provided is a method for delivery of abacavir sulfate to a patient comprising administering to the patient a composition comprising a polypeptide and abacavir sulfate covalently attached to the polypeptide. Also provided is a method for
15 protecting abacavir sulfate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of abacavir sulfate from a composition comprising covalently attaching it to a polypeptide.

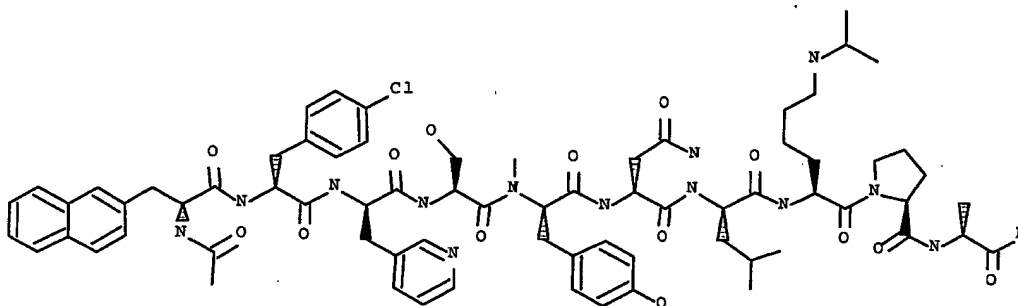
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ABARELIX AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to abarelix, as well as methods for protecting and administering abarelix. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Abarelix is a known pharmaceutical agent that is used in the treatment of prostate cancer, acting as a gonadotropin-releasing hormone antagonist. Its chemical name is N-acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-N-methyl-L-tyrosyl-D-asparaginy-L-N6-(1-methylethyl)-L-lysyl-L-prolyl-D-alaninamide. Abarelix is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is as follows:



CW002P

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release

CW002P

through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide

CW002P

linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (abarelix) to a polymer of peptides or amino acids. The invention is distinguished from the above mentioned technologies by virtue of covalently attaching abarelix to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection.

In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising abarelix microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and abarelix covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,

CW002P

(ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Abarelix preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting abarelix from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering abarelix to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the

CW002P

polypeptide. In a preferred embodiment, abarelix is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, abarelix is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a

5 microencapsulating agent and abarelix is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, abarelix is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, abarelix is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant

10 covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method

15 comprises the steps of:

- (a) attaching abarelix to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- 20 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, abarelix and a second active agent can be copolymerized in step (c). In another

25 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,

30 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a

CW002P

carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

5 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

10 The present invention provides several benefits for active agent delivery. First, the invention can stabilize abarelix and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of abarelix. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted
15 delivery of active agents to specific sites of action.

The composition of the invention comprises abarelix covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a
20 heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and
25 turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW002P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW002P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW002P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW002P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, abarelix is covalently attached to the polypeptide via the free alcohol group or, alternatively, through one of its amino groups.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW002P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

5 The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

10 There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the
15 transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active
20 agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the
25 catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-abarelix conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW002P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW002P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW002P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW002P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 abarelix covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein abarelix is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein abarelix is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing abarelix from said composition in a pH-dependent manner.

15 19. A method for protecting abarelix from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of abarelix from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching abarelix to said polypeptide.

20 21. A method for delivering abarelix to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
abarelix covalently attached to said polypeptide.

25 22. The method of claim 21 wherein abarelix is released from said composition by an enzyme-catalyzed release.

CW002P

23. The method of claim 21 wherein abarelix is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

A composition comprising a polypeptide and abarelix covalently attached to the polypeptide. Also provided is a method for delivery of abarelix to a patient comprising administering to the patient a composition comprising a polypeptide and abarelix
15 covalently attached to the polypeptide. Also provided is a method for protecting abarelix from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of abarelix from a composition comprising covalently attaching it to the polypeptide.

CW003P

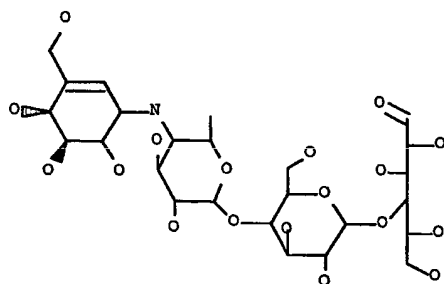
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ACARBOSE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to acarbose, as well as methods for protecting and administering acarbose. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Acarbose is a known pharmaceutical agent that is used in the treatment of type II diabetes. Its chemical name is O-4,6-dideoxy-4-[[[1S-(1 α ,4 α ,5 β ,6 α)]-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]- α -D-glucopyranosyl-(1-4)-O- α -D-glucopyranosyl-(1-4)-D-glucose. Its structure is as follows:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;

CW003P

and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

CW003P

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

CW003P

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (acarbose) to a polymer of peptides or amino acids. The invention is distinguished from the above mentioned technologies by virtue of covalently attaching acarbose to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising acarbose microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and acarbose covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

acarbose preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting acarbose from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering acarbose to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, acarbose is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, acarbose is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release.

CW003P

In another preferred embodiment, the composition further comprises a microencapsulating agent and acarbose is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, acarbose is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, acarbose is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

10 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching acarbose to a side chain of an amino acid to form an active agent/amino acid complex;
- 15 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, acarbose and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

CW003P

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000,
5 incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize acarbose and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of acarbose. Furthermore,
10 active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Acarbose is the subject of U.S. Patent Number 4,904,769, herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises acarbose covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
20 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
25 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW003P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW003P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW003P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW003P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW003P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, acarbose is covalently attached to the polypeptide via any of the free hydroxyl groups.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW003P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-acarbose conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW003P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

5 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW003P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 acarbose covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein acarbose is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein acarbose is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing acarbose from said composition in a pH-dependent manner.

15 19. A method for protecting acarbose from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of acarbose from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching acarbose to said polypeptide.

20 21. A method for delivering acarbose to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
acarbose covalently attached to said polypeptide.

25 22. The method of claim 21 wherein acarbose is released from said composition by an enzyme-catalyzed release.

CW003P

23. The method of claim 21 wherein acarbose is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

15 A composition comprising a polypeptide and acarbose covalently attached to the polypeptide. Also provided is a method for delivery of acarbose to a patient comprising administering to the patient a composition comprising a polypeptide and acarbose covalently attached to the polypeptide. Also provided is a method for protecting acarbose from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of acarbose from a composition comprising covalently
20 attaching it to the polypeptide.

**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
ACETAMINOPHEN AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to acetaminophen, as well as methods for protecting and administering acetaminophen. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Acetaminophen is a known pharmaceutical agent that is used in the treatment of minor aches and pains. Its chemical name is N-acetyl-p-aminophenol. Acetaminophen is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art.

 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another

CW004P

invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

CW004P

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

CW004P

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (acetaminophen) to a polymer of peptides or amino acids. The invention is distinguished from the above mentioned technologies by virtue of covalently attaching acetaminophen to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising acetaminophen microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and acetaminophen covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

acetaminophen preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

CW004P

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting acetaminophen from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering acetaminophen to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, acetaminophen is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, acetaminophen is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and acetaminophen is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, acetaminophen is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, acetaminophen is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

CW004P

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching acetaminophen to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, acetaminophen and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize acetaminophen and prevent its digestion in the stomach. In

CW004P

addition, the pharmacologic effect can be prolonged by delayed release of acetaminophen. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

5 The composition of the invention comprises acetaminophen covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
10 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
15 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
20 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
25 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior

CW004P

and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are
5 “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

10 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only
15 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

20 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For
25 instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will

CW004P

ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
5 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
10 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
15 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
20 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate
25 weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the

CW004P

jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

5

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order

CW004P

to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
5 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

10 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
15 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
20 any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
25 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
30 polypeptides through a spacer or linker on the pendant group, which is terminated,

CW004P

preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, acetaminophen is covalently attached to the polypeptide via its hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known

CW004P

intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
5 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
10 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

15 Preferably, the resultant peptide-acetaminophen conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

20 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or
25 dialysis.

Amine/C-terminus conjugation

CW004P

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

CW004P

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

5 precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product,

10 which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and

15 filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

20

CW004P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 acetaminophen covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein acetaminophen is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW004P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein acetaminophen is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing acetaminophen from said composition in a pH-dependent manner.

15 19. A method for protecting acetaminophen from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of acetaminophen from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching acetaminophen to said polypeptide.

20 21. A method for delivering acetaminophen to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
acetaminophen covalently attached to said polypeptide.

25 22. The method of claim 21 wherein acetaminophen is released from said composition by an enzyme-catalyzed release.

CW004P

23. The method of claim 21 wherein acetaminophen is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW005P

**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
ACETAMINOPHEN AND CODEINE AND METHODS OF MAKING AND
USING SAME**

5 FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to both acetaminophen and codeine, as well as methods for protecting and administering acetaminophen and codeine together. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA),
10 has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Acetaminophen is a known pharmaceutical agent that is used in the treatment of minor aches and pains. Its chemical name is N-acetyl-p-aminophenol. It is often used in combination with codeine, whose chemical name is 7,8-didehydro-4,5- α -epoxy-3-methoxy-17-methylmephorminan-6 α -ol. Both are commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art.

20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical
25 compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken

CW005P

under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even
5 reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of
10 cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release
20 through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several
25 shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent

CW005P

in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

5 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
10 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
15 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
20 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

 It is also important to control the molecular weight, molecular size and particle
25 size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.
30 Particle size not only becomes a problem with injectable drugs, as in the HAR

CW005P

application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
5 (acetaminophen and codeine) to a polymer of peptides or amino acids. The invention is distinguished from the above mentioned technologies by virtue of covalently attaching acetaminophen and codeine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily
10 in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release
15 mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising acetaminophen and codeine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and acetaminophen and codeine covalently attached to the polypeptide. Preferably, the
20 polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 acetaminophen and codeine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached

CW005P

to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting acetaminophen and codeine from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering acetaminophen and codeine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently
20 attached to the polypeptide. In a preferred embodiment, acetaminophen and codeine are released from the composition by an enzyme-catalyzed release. In another preferred embodiment, acetaminophen and codeine are released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred
25 embodiment, the composition further comprises a microencapsulating agent and acetaminophen and codeine are released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, acetaminophen and codeine are released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, acetaminophen and codeine are released from the composition in a sustained release. In yet another preferred embodiment, the

CW005P

composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching acetaminophen to a side chain of an amino acid to form an active agent/amino acid complex;

10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, acetaminophen and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW005P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize acetaminophen and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of acetaminophen. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises acetaminophen covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW005P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

CW005P

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

25

CW005P

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW005P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

CW005P

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, acetaminophen and codeine are covalently attached to the polypeptide via its hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

CW005P

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-acetaminophen conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW005P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW005P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 acetaminophen covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein acetaminophen and codeine are
covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW005P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein acetaminophen and codeine are conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing acetaminophen from said composition in a pH-dependent manner.

15 19. A method for protecting acetaminophen from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of acetaminophen from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching acetaminophen to said polypeptide.

20 21. A method for delivering acetaminophen to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
acetaminophen covalently attached to said polypeptide.

25 22. The method of claim 21 wherein acetaminophen and codeine are released from said composition by an enzyme-catalyzed release.

CW005P

23. The method of claim 21 wherein acetaminophen and codeine are released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW005P

Abstract

A composition comprising a polypeptide and acetaminophen covalently attached to the polypeptide. Also provided is a method for delivery of acetaminophen to a patient comprising administering to the patient a composition comprising a polypeptide and
5 acetaminophen covalently attached to the polypeptide. Also provided is a method for protecting acetaminophen from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of acetaminophen from a composition comprising covalently attaching it to the polypeptide.

CW006P

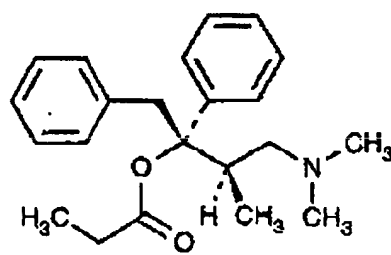
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING PROPOXYPHENE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to propoxyphene, as well as methods for protecting and administering propoxyphene. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Propoxyphene is a known pharmaceutical agent that is used in the treatment of pain. It is a mild narcotic analgesic. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is as follows:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

CW006P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW006P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW006P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (propoxyphene) to a polymer of peptides or amino acids. The invention is distinguished from the above mentioned technologies by virtue of covalently attaching propoxyphene to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising propoxyphene microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and propoxyphene covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

propoxyphene preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW006P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting propoxyphene from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering propoxyphene to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, propoxyphene is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, propoxyphene is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and propoxyphene is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, propoxyphene is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, propoxyphene is

CW006P

released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug
5 conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching propoxyphene to a side chain of an amino acid to form an active
10 agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, propoxyphene and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize propoxyphene and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of propoxyphene. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises propoxyphene covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW006P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

CW006P

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

CW006P

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW006P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

CW006P

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, propoxyphene is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW006P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-propoxyphene conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW006P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 propoxyphene covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein propoxyphene is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein propoxyphene is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing propoxyphene from said composition in a pH-dependent manner.

15 19. A method for protecting propoxyphene from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of propoxyphene from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching propoxyphene to said polypeptide.

20 21. A method for delivering propoxyphene to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
propoxyphene covalently attached to said polypeptide.

25 22. The method of claim 21 wherein propoxyphene is released from said composition by an enzyme-catalyzed release.

CW006P

23. The method of claim 21 wherein propoxyphene is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and propoxyphene covalently attached to the polypeptide. Also provided is a method for delivery of propoxyphene to a patient comprising administering to the patient a composition comprising a polypeptide and propoxyphene covalently attached to the polypeptide. Also provided is a method for protecting propoxyphene from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of propoxyphene from a composition comprising covalently attaching it to the polypeptide.

**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
ACETYLSALICYLIC ACID AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to acetylsalicylic acid, as well as methods for protecting and administering acetylsalicylic acid. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Acetylsalicylic acid is a known pharmaceutical agent that is used in the treatment
15 of minor aches and pains. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art.

 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

 Active agent delivery systems are often critical for the effective delivery of a
25 biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf

CW007P

life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

CW007P

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (acetylsalicylic acid) to a polymer of peptides or amino acids. The invention is distinguished from the above mentioned technologies by virtue of covalently attaching
5 acetylsalicylic acid to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry
10 into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising acetylsalicylic acid microencapsulated by a polypeptide.

15 The invention provides a composition comprising a polypeptide and acetylsalicylic acid covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic
20 amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

acetylsalicylic acid preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide.
25 In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

CW007P

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting acetylsalicylic acid from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering acetylsalicylic acid to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, acetylsalicylic acid is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, acetylsalicylic acid is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and acetylsalicylic acid is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, acetylsalicylic acid is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, acetylsalicylic acid is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

CW007P

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching acetylsalicylic acid to a side chain of an amino acid to form an active agent/amino acid complex;
 - (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
 - (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).
- In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, acetylsalicylic acid and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

- It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
- The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- The present invention provides several benefits for active agent delivery. First, the invention can stabilize acetylsalicylic acid and prevent its digestion in the stomach.

CW007P

In addition, the pharmacologic effect can be prolonged by delayed release of acetylsalicylic acid. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

5 The composition of the invention comprises acetylsalicylic acid covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of
10 one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
15 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
20 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
25 model for determining forces contributing to protein stability is the solid reference state.

 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior

CW007P

and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are
5 “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

10 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only
15 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

20 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For
25 instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will

CW007P

ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
5 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
10 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
15 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
20 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate
25 weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the

CW007P

jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

5

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order

CW007P

to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
5 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

10 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
15 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
20 any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
25 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
30 polypeptides through a spacer or linker on the pendant group, which is terminated,

CW007P

preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
5 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
10 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, acetylsalicylic acid is covalently attached to the polypeptide via the hydroxy group.

The polypeptide carrier can be prepared using conventional techniques. A
15 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG)
20 and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
25 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known

CW007P

intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
5 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
10 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

15 Preferably, the resultant peptide-acetylsalicylic acid conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

20 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or
25 dialysis.

Amine/C-terminus conjugation

CW007P

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

CW007P

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for
5 several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes
10 homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically
15 overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of
20 equivalents of the claims and without departing from the spirit of the invention.

CW007P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 acetylsalicylic acid covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein acetylsalicylic acid is covalently attached
to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW007P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein acetylsalicylic acid is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing acetylsalicylic acid from said composition in a pH-dependent manner.

15 19. A method for protecting acetylsalicylic acid from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of acetylsalicylic acid from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching acetylsalicylic acid to said polypeptide.

20 21. A method for delivering acetylsalicylic acid to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
acetylsalicylic acid covalently attached to said polypeptide.

25 22. The method of claim 21 wherein acetylsalicylic acid is released from said composition by an enzyme-catalyzed release.

CW007P

23. The method of claim 21 wherein acetylsalicylic acid is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW007P

Abstract

A composition comprising a polypeptide and acetylsalicylic acid covalently attached to the polypeptide. Also provided is a method for delivery of acetylsalicylic acid to a patient comprising administering to the patient a composition comprising a polypeptide and acetylsalicylic acid covalently attached to the polypeptide. Also provided is a method for protecting acetylsalicylic acid from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of acetylsalicylic acid from a composition comprising covalently attaching it to the polypeptide.

CW008P

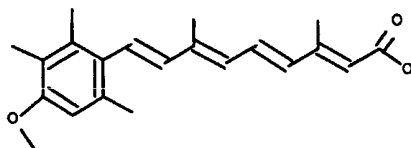
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ACITRETIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to acitretin, as well as methods for protecting and administering acitretin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Acitretin is a known pharmaceutical agent that is used in the treatment of
15 psoriasis. Its chemical name is (all-E)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical
25 compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW008P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW008P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (acitretin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching acitretin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising acitretin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and acitretin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Acitretin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW008P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting acitretin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering acitretin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, acitretin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, acitretin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and acitretin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, acitretin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, acitretin is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

 (a) attaching acitretin to a side chain of an amino acid to form an active agent/amino acid complex;

10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, acitretin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize acitretin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of acitretin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises acitretin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW008P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

CW008P

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.
- 25

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, acitretin is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

CW008P

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-acitretin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 acitretin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein acitretin is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW008P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein acitretin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing acitretin from said composition in a pH-dependent manner.

15 19. A method for protecting acitretin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of acitretin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching acitretin to said polypeptide.

20 21. A method for delivering acitretin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
acitretin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein acitretin is released from said composition by an enzyme-catalyzed release.

CW008P

23. The method of claim 21 wherein acitretin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and acitretin covalently attached to the polypeptide. Also provided is a method for delivery of acitretin to a patient comprising administering to the patient a composition comprising a polypeptide and acitretin

5 covalently attached to the polypeptide. Also provided is a method for protecting acitretin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of acitretin from a composition comprising covalently attaching it to the polypeptide.

**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
ACTIVATED PROTEIN C AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to activated protein C, as well as methods for protecting and administering activated protein C. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Activated protein C is a known pharmaceutical agent that is used in the treatment
15 of blood clots. Its structure is well known and it is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art.

 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another

invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

CW009P

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (activated protein C) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching activated protein C to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising activated protein C microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and activated protein C covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Activated protein C preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

CW009P

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting activated protein C from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering activated protein C to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, activated protein C is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, activated protein C is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and activated protein C is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, activated protein C is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, activated protein C is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

CW009P

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching activated protein C to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, activated protein C and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize activated protein C and prevent its digestion in the stomach.

CW009P

In addition, the pharmacologic effect can be prolonged by delayed release of activated protein C. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

5 The composition of the invention comprises activated protein C covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of
10 one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
15 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
20 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
25 model for determining forces contributing to protein stability is the solid reference state.

 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior

CW009P

and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are
5 “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

10 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only
15 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

20 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For
25 instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will

CW009P

ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
5 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
10 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
15 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
20 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate
25 weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the

CW009P

jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

5

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order

CW009P

to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
5 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

10 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
15 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
20 any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
25 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
30 polypeptides through a spacer or linker on the pendant group, which is terminated,

CW009P

preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, activated protein C is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known

CW009P

intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
5 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
10 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

15 Preferably, the resultant peptide-activated protein C conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW009P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW009P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

5 What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
activated protein C covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
- 10 3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
15 synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
- 20 8. The composition of claim 1 wherein activated protein C is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.

CW009P

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

5 12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

10 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

15 17. The composition of claim 1 wherein activated protein C is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing activated protein C from said composition in a pH-dependent manner.

20 19. A method for protecting activated protein C from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of activated protein C from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching activated protein C to said polypeptide.

25 21. A method for delivering activated protein C to a patient comprising administering to said patient a composition comprising:

a polypeptide; and
activated protein C covalently attached to said polypeptide.

22. The method of claim 21 wherein activated protein C is released from said composition by an enzyme-catalyzed release.

5 23. The method of claim 21 wherein activated protein C is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an
10 adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and activated protein C covalently attached to the polypeptide. Also provided is a method for delivery of activated protein C to a patient comprising administering to the patient a composition comprising a

5 polypeptide and activated protein C covalently attached to the polypeptide. Also provided is a method for protecting activated protein C from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of activated protein C from a composition comprising covalently attaching it to the polypeptide.

CW010P

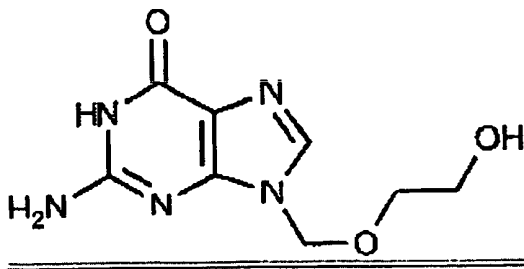
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ACYCLOVIR
AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to acyclovir, as well as methods for protecting and administering acyclovir. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Acyclovir is a known pharmaceutical agent that is an antiviral drug used in the
15 treatment of herpes simplex viruses. Acyclovir is both commercially available and readily manufactured using public synthetic schemes by those of ordinary skill in the art. Its chemical name is 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one. Its structure is:



20

 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;

CW010P

and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

CW010P

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

CW010P

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (acyclovir) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching acyclovir to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising acyclovir microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and acyclovir covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

CW010P

heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Acyclovir preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a
5 carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-
10 terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
15 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
20 composition in a pH-dependent manner.

The invention also provides a method for protecting acyclovir from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering acyclovir to a patient, the patient being a human or a non-human animal, comprising administering to the patient a
25 composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, acyclovir is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, acyclovir is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release.

CW010P

In another preferred embodiment, the composition further comprises a microencapsulating agent and acyclovir is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, acyclovir is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, acyclovir is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

10 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching acyclovir to a side chain of an amino acid to form an active agent/amino acid complex;
- 15 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, acyclovir and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

20
25
30

CW010P

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize acyclovir and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of acyclovir. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises acyclovir covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW010P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

- 5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
- 10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
- 15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

- Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
- 20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
- 25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW010P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW010P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
30

CW010P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, acyclovir is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW010P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-acyclovir conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW010P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 acyclovir covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein acyclovir is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.
- 5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein acyclovir is conformationally protected by folding of said polypeptide about said active agent.
18. The composition of claim 1 wherein said polypeptide is capable of releasing acyclovir from said composition in a pH-dependent manner.
- 15 19. A method for protecting acyclovir from degradation comprising covalently attaching said active agent to a polypeptide.
20. A method for controlling release of acyclovir from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching acyclovir to said polypeptide.
- 20 21. A method for delivering acyclovir to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
acyclovir covalently attached to said polypeptide.
- 25 22. The method of claim 21 wherein acyclovir is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein acyclovir is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW010P

Abstract

A composition comprising a polypeptide and acyclovir covalently attached to the polypeptide. Also provided is a method for delivery of acyclovir to a patient comprising administering to the patient a composition comprising a polypeptide and acyclovir covalently attached to the polypeptide. Also provided is a method for protecting acyclovir from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of acyclovir from a composition comprising covalently attaching it to the polypeptide.

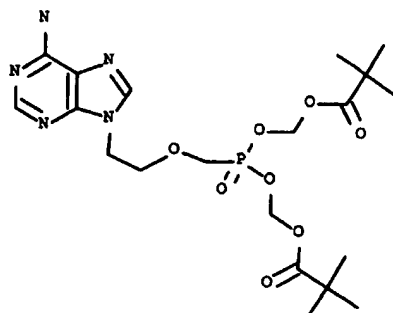
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
ADEFOVIR DIPIVOXIL AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to adefovir dipivoxil, as well as methods for protecting and administering adefovir dipivoxil. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Adefovir dipivoxil is a known pharmaceutical agent that is used in the treatment
15 of AIDS. Its chemical name is [[[2-(6-amino-9H-purin-9-yl)ethoxy]methyl]phosphinylidene]bis(oxymethylene)-2,2-dimethylpropanoic acid. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

CW011P

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW011P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW011P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- 10 The present invention provides covalent attachment of the active agent (adefovir dipivoxil) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching adefovir dipivoxil to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
- 15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising adefovir dipivoxil microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and adefovir dipivoxil covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,
- 25 (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW011P

Adefovir dipivoxil preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting adefovir dipivoxil from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering adefovir dipivoxil to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, adefovir dipivoxil is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, adefovir dipivoxil is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and adefovir dipivoxil is released from the composition by dissolution of the microencapsulating agent. In another preferred

CW011P

embodiment, adefovir dipivoxil is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, adefovir dipivoxil is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching adefovir dipivoxil to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, adefovir dipivoxil and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

CW011P

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000,
5 incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize adefovir dipivoxil and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of adefovir
10 dipivoxil. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Adefovir dipivoxil is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art.

15 The composition of the invention comprises adefovir dipivoxil covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of
20 one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
25 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW011P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW011P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW011P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW011P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW011P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, adefovir dipivoxil is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW011P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-adeфовir dipivoxil conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW011P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW011P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW011P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 adefovir dipivoxil covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein adefovir dipivoxil is covalently attached
to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW011P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein adefovir dipivoxil is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing adefovir dipivoxil from said composition in a pH-dependent manner.

15 19. A method for protecting adefovir dipivoxil from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of adefovir dipivoxil from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching adefovir dipivoxil to said polypeptide.

20 21. A method for delivering adefovir dipivoxil to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
adefovir dipivoxil covalently attached to said polypeptide.

25 22. The method of claim 21 wherein adefovir dipivoxil is released from said composition by an enzyme-catalyzed release.

CW011P

23. The method of claim 21 wherein adefovir dipivoxil is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

 A composition comprising a polypeptide and adefovir dipivoxil covalently attached to the polypeptide. Also provided is a method for delivery of adefovir dipivoxil to a patient comprising administering to the patient a composition comprising a polypeptide and adefovir dipivoxil covalently attached to the polypeptide. Also provided
15 is a method for protecting adefovir dipivoxil from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of adefovir dipivoxil from a composition comprising covalently attaching it to the polypeptide.

CW012P

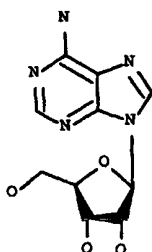
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ADENOSINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to adenosine, as well as methods for protecting and administering adenosine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Adenosine is a known pharmaceutical agent that is used as a coronary vasodilator.
15 Its chemical name is 9- α -D-ribofuranosyl-9H-purin-6-amine. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW012P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW012P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW012P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (adenosine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching adenosine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising adenosine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and adenosine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Adenosine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW012P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting adenosine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering adenosine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, adenosine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, adenosine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and adenosine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, adenosine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, adenosine is released from the composition in a sustained release.

CW012P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching adenosine to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, adenosine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW012P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize adenosine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of adenosine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Adenosine is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art.

The composition of the invention comprises adenosine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW012P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW012P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW012P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW012P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, adenosine is covalently attached to the polypeptide via the ribose hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW012P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-adenosine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW012P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW012P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW012P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 adenosine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
15 one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein adenosine is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
10. The composition of claim 9 wherein said microencapsulating agent is
20 selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW012P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein adenosine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing adenosine from said composition in a pH-dependent manner.

15 19. A method for protecting adenosine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of adenosine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching adenosine to said polypeptide.

20 21. A method for delivering adenosine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
adenosine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein adenosine is released from said composition by an enzyme-catalyzed release.

CW012P

23. The method of claim 21 wherein adenosine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW012P

Abstract

A composition comprising a polypeptide and adenosine covalently attached to the polypeptide. Also provided is a method for delivery of adenosine to a patient comprising administering to the patient a composition comprising a polypeptide and adenosine covalently attached to the polypeptide. Also provided is a method for protecting adenosine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of adenosine from a composition comprising covalently attaching it to the polypeptide.

CW013P

**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
ADRENOCORTICOTROPIC HORMONE AND METHODS OF MAKING AND
USING SAME**

5 FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to adrenocorticotrophic hormone, as well as methods for protecting and administering adrenocorticotrophic hormone. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has
10 the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Adrenocorticotrophic hormone is a known pharmaceutical agent that is useful for the diagnosis of Addison's disease and other conditions in which the functionality of the adrenal cortex is to be determined. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art.

The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

CW013P

markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

5 Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres,
10 liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

 Active agent delivery systems also provide the ability to control the release of the
15 active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified
20 amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

 Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the
25 microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some

CW013P

technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR

CW013P

application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
5 (adrenocorticotrophic hormone) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching adrenocorticotrophic hormone to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily
10 in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release
15 mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising adrenocorticotrophic hormone microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and adrenocorticotrophic hormone covalently attached to the polypeptide. Preferably, the
20 polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Adrenocorticotrophic hormone preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is

CW013P

covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting adrenocorticotrophic hormone from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering adrenocorticotrophic hormone to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently
20 attached to the polypeptide. In a preferred embodiment, adrenocorticotrophic hormone is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, adrenocorticotrophic hormone is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and
25 adrenocorticotrophic hormone is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, adrenocorticotrophic hormone is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, adrenocorticotrophic hormone is released from the composition in a sustained release. In yet another preferred embodiment, the

CW013P

composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching adrenocorticotrophic hormone to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, adrenocorticotrophic hormone and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident
20 intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred
25 embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

CW013P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize adrenocorticotrophic hormone and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of adrenocorticotrophic hormone. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites
10 of action.

The composition of the invention comprises adrenocorticotrophic hormone covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a
15 synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW013P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW013P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW013P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW013P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
30

CW013P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, adrenocorticotrophic hormone is covalently attached to the polypeptide via an amide bond.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW013P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-adrenocorticotrophic hormone conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW013P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW013P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW013P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 adrenocorticotropic hormone covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein adrenocorticotropic hormone is
covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein adrenocorticotrophic hormone is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing adrenocorticotrophic hormone from said composition in a pH-dependent manner.

15 19. A method for protecting adrenocorticotrophic hormone from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of adrenocorticotrophic hormone from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching adrenocorticotrophic hormone to said polypeptide.

20 21. A method for delivering adrenocorticotrophic hormone to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
adrenocorticotrophic hormone covalently attached to said polypeptide.

25 22. The method of claim 21 wherein adrenocorticotrophic hormone is released from said composition by an enzyme-catalyzed release.

CW013P

23. The method of claim 21 wherein adrenocorticotrophic hormone is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

10 A composition comprising a polypeptide and adrenocorticotrophic hormone covalently attached to the polypeptide. Also provided is a method for delivery of adrenocorticotrophic hormone to a patient comprising administering to the patient a composition comprising a polypeptide and adrenocorticotrophic hormone covalently attached to the polypeptide. Also provided is a method for protecting adrenocorticotrophic
15 hormone from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of adrenocorticotrophic hormone from a composition comprising covalently attaching it to the polypeptide.

CW014P

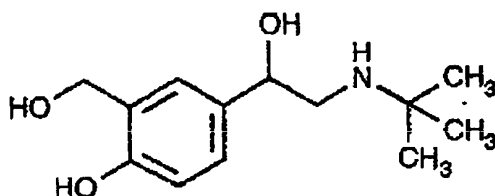
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ALBUTEROL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to albuterol, as well as methods for protecting and administering albuterol. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Albuterol is a known pharmaceutical agent that is used for the symptomatic management of bronchospasm in patients with reversible, obstructive airway disease. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

CW014P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW014P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some
5 technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically
10 require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the
15 gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The
20 released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that
25 incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly
30 or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW014P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (albuterol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching albuterol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising albuterol microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and albuterol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Albuterol preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting albuterol from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering albuterol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, albuterol is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, albuterol is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and albuterol is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, albuterol is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, albuterol is released from the composition in a sustained release.

CW014P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching albuterol to a side chain of an amino acid to form an active agent/amino acid complex;

10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, albuterol and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW014P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize albuterol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of albuterol. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises albuterol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW014P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

CW014P

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.
- 25

CW014P

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW014P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

CW014P

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, albuterol is covalently attached to the polypeptide via one of the hydroxyl groups.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

CW014P

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-albuterol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW014P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW014P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 albuterol covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein albuterol is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein albuterol is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing albuterol from said composition in a pH-dependent manner.

15 19. A method for protecting albuterol from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of albuterol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching albuterol to said polypeptide.

20 21. A method for delivering albuterol to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
albuterol covalently attached to said polypeptide.

25 22. The method of claim 21 wherein albuterol is released from said composition by an enzyme-catalyzed release.

CW014P

23. The method of claim 21 wherein albuterol is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and albuterol covalently attached to the polypeptide. Also provided is a method for delivery of albuterol to a patient comprising administering to the patient a composition comprising a polypeptide and albuterol covalently attached to the polypeptide. Also provided is a method for protecting albuterol from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of albuterol from a composition comprising covalently attaching it to the polypeptide.

5

CW015P

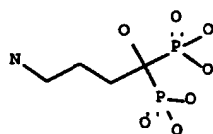
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ALENDRONATE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to alendronate, as well as methods for protecting and administering alendronate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Alendronate is a known pharmaceutical agent that is used for controlling
15 osteoporosis in men. Its chemical name is (4-amino-1-hydroxybutylidene)bisphosphonic acid. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

CW015P

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

CW015P

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

CW015P

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (alendronate) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching alendronate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

 Alternatively, the present invention provides a pharmaceutical composition comprising alendronate microencapsulated by a polypeptide.

 The invention provides a composition comprising a polypeptide and alendronate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Alendronate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

CW015P

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting alendronate from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering alendronate to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, alendronate is released from the composition by
20 an enzyme-catalyzed release. In another preferred embodiment, alendronate is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and alendronate is released from the composition by
25 dissolution of the microencapsulating agent. In another preferred embodiment, alendronate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, alendronate is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and

CW015P

release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching alendronate to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, alendronate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

CW015P

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize alendronate and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of alendronate.

5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Alendronate is the subject of U.S. Patent Numbers 4,621,077, 5,358,941, 5,681,950, 5,804,570, 5,849,726, 6,008,207, and 6,090,410, herein incorporated by
10 reference, which describe how to make that drug.

The composition of the invention comprises alendronate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a
15 heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and
20 turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on
25 the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the

CW015P

protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van
5 der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect
refers to the energetic consequences of removing apolar groups from the protein interior
and exposing them to water. Comparing the energy of amino acid hydrolysis with
protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular
bonds are formed at the expense of hydrogen bonds with water. Water molecules are
“pushed out” of the packed, hydrophobic protein core. All of these forces combine and
contribute to the overall stability of the folded protein where the degree to which ideal
packing occurs determines the degree of relative stability of the protein. The result of
15 maximum packing is to produce a center of residues or hydrophobic core that has
maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
peptide, it would require energy to unfold the peptide before the drug can be released.
The unfolding process requires overcoming the hydrophobic effect by hydrating the
20 amino acids or achieving the melting temperature of the protein. The heat of hydration is
a destabilization of a protein. Typically, the folded state of a protein is favored by only
5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
often observed prior to the onset of irreversible chemical or conformation processes.
25 Moreover, protein conformation generally controls the rate and extent of deleterious
chemical reactions.

Conformational protection of active agents by proteins depends on the stability of
the protein's folded state and the thermodynamics associated with the agent's

CW015P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW015P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW015P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
30

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, alendronate is covalently attached to the polypeptide via the hydroxyl or phosphate groups.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW015P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-alendronate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW015P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW015P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 alendronate covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein alendronate is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein alendronate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing alendronate from said composition in a pH-dependent manner.

15 19. A method for protecting alendronate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of alendronate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching alendronate to said polypeptide.

20 21. A method for delivering alendronate to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
alendronate covalently attached to said polypeptide.

25 22. The method of claim 21 wherein alendronate is released from said composition by an enzyme-catalyzed release.

CW015P

23. The method of claim 21 wherein alendronate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW015P

Abstract

A composition comprising a polypeptide and alendronate covalently attached to the polypeptide. Also provided is a method for delivery of alendronate to a patient comprising administering to the patient a composition comprising a polypeptide and alendronate covalently attached to the polypeptide. Also provided is a method for
5 protecting alendronate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of alendronate from a composition comprising covalently attaching it to the polypeptide.

CW016P

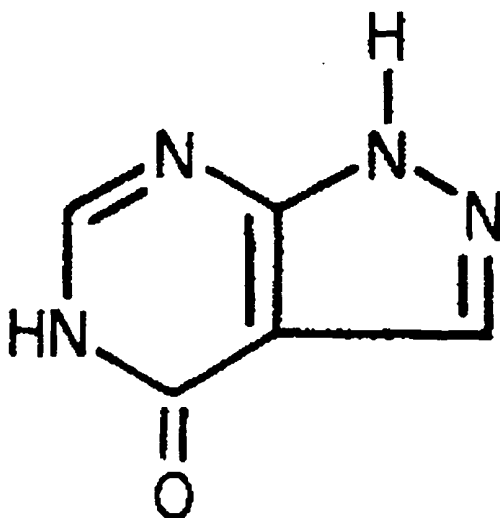
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ALLOPURINAL
AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to allopurinal, as well as methods for protecting and administering allopurinal. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Allopurinal is a known pharmaceutical agent that is is a xanthine oxidase inhibitor
15 used in the treatment of gout and selected hyperuricemias. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



CW016P

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release

CW016P

through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide

CW016P

linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (allopurinol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching allopurinol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising allopurinol microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and allopurinol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,

CW016P

(ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Allopurinol preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting allopurinol from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering allopurinol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the

CW016P

polypeptide. In a preferred embodiment, allopurinol is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, allopurinol is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a
5 microencapsulating agent and allopurinol is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, allopurinol is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, allopurinol is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an
10 adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
15 comprises the steps of:

(a) attaching allopurinol to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

20 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, allopurinol and a second active agent can be copolymerized in step (c). In another
25 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
30 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a

carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize allopurinol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of allopurinol. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises allopurinol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW016P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW016P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW016P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW016P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

- 5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

- The invention also provides a method of imparting the same mechanism of action
10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

- The active agent can be covalently attached to the N-terminus, the C-terminus or
20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

- 25 In the present invention, allopurinol is covalently attached to the polypeptide via its -NH group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW016P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-allopurinol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW016P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW016P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

5 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 allopurinal covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein allopurinal is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW016P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein allopurinol is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing allopurinol from said composition in a pH-dependent manner.

15 19. A method for protecting allopurinol from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of allopurinol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching allopurinol to said polypeptide.

20 21. A method for delivering allopurinol to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
allopurinol covalently attached to said polypeptide.

25 22. The method of claim 21 wherein allopurinol is released from said composition by an enzyme-catalyzed release.

CW016P

23. The method of claim 21 wherein allopurinol is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

- 10 A composition comprising a polypeptide and allopurinol covalently attached to the polypeptide. Also provided is a method for delivery of allopurinol to a patient comprising administering to the patient a composition comprising a polypeptide and allopurinol covalently attached to the polypeptide. Also provided is a method for protecting allopurinol from degradation comprising covalently attaching it to a
- 15 polypeptide. Also provided is a method for controlling release of allopurinol from a composition comprising covalently attaching it to the polypeptide.

**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ALPHA 1
PROTEINASE INHIBITOR AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises
a polypeptide that is preferably covalently attached to Alpha 1 proteinase inhibitor, as
well as methods for protecting and administering Alpha 1 proteinase inhibitor. This
novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has
the benefit of taking a known effective pharmaceutical agent that is both well studied and
10 occupies a known segment of the pharmaceutical market, and combining it with a carrier
compound that enhances the usefulness of the pharmaceutical agent without
compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Alpha 1 proteinase inhibitor is a known pharmaceutical agent that is used in the
15 treatment of emphysema. It is a natural product isolated from human blood, using
methods known to those of ordinary skill in the art.

 The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical
compound may contain one or more of the following: another active pharmaceutical
agent, an adjuvant, or an inhibitor.

 Active agent delivery systems are often critical for the effective delivery of a
25 biologically active agent (active agent) to the appropriate target. The importance of these
systems becomes magnified when patient compliance and active agent stability are taken
under consideration. For instance, one would expect patient compliance to increase
markedly if an active agent is administered orally in lieu of an injection or another
invasive technique. Increasing the stability of the active agent, such as prolonging shelf

CW017P

life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

CW017P

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (Alpha 1 proteinase inhibitor) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching

5 Alpha 1 proteinase inhibitor to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide.

10 Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition

15 comprising Alpha 1 proteinase inhibitor microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and Alpha 1 proteinase inhibitor covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a

20 homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Alpha 1 proteinase inhibitor preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active

25 agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet

CW017P

another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting Alpha 1 proteinase inhibitor from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering Alpha 1 proteinase inhibitor to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, Alpha 1 proteinase inhibitor is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, Alpha 1 proteinase inhibitor is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and Alpha 1 proteinase inhibitor is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, Alpha 1 proteinase inhibitor is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, Alpha 1 proteinase inhibitor is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The

adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

(a) attaching Alpha 1 proteinase inhibitor to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, Alpha 1 proteinase inhibitor and a second active agent can be copolymerized in
15 step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine,
20 asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid
25 functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000,
30 incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize Alpha 1 proteinase inhibitor and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of Alpha 1 proteinase inhibitor. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises Alpha 1 proteinase inhibitor covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

CW017P

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.
- 25

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW017P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

CW017P

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the
25 polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-Alpha 1 proteinase inhibitor conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

25 **Acid/N-terminus conjugation**

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be

stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

- 5 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

10 Alcohol/N-Terminus Conjugation

- In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
15 added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
20 solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- 25 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

CW017P

several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- 20 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW017P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 Alpha 1 proteinase inhibitor covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein Alpha 1 proteinase inhibitor is covalently
attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein Alpha 1 proteinase inhibitor is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing Alpha 1 proteinase inhibitor from said composition in a pH-dependent manner.

15 19. A method for protecting Alpha 1 proteinase inhibitor from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of Alpha 1 proteinase inhibitor from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching Alpha 1 proteinase inhibitor to said polypeptide.

20 21. A method for delivering Alpha 1 proteinase inhibitor to a patient comprising administering to said patient a composition comprising:

 a polypeptide; and

 Alpha 1 proteinase inhibitor covalently attached to said polypeptide.

25 22. The method of claim 21 wherein Alpha 1 proteinase inhibitor is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein Alpha 1 proteinase inhibitor is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and Alpha 1 proteinase inhibitor covalently attached to the polypeptide. Also provided is a method for delivery of Alpha 1 proteinase inhibitor to a patient comprising administering to the patient a composition

5 comprising a polypeptide and Alpha 1 proteinase inhibitor covalently attached to the polypeptide. Also provided is a method for protecting Alpha 1 proteinase inhibitor from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of Alpha 1 proteinase inhibitor from a composition comprising covalently attaching it to the polypeptide.

CW018P

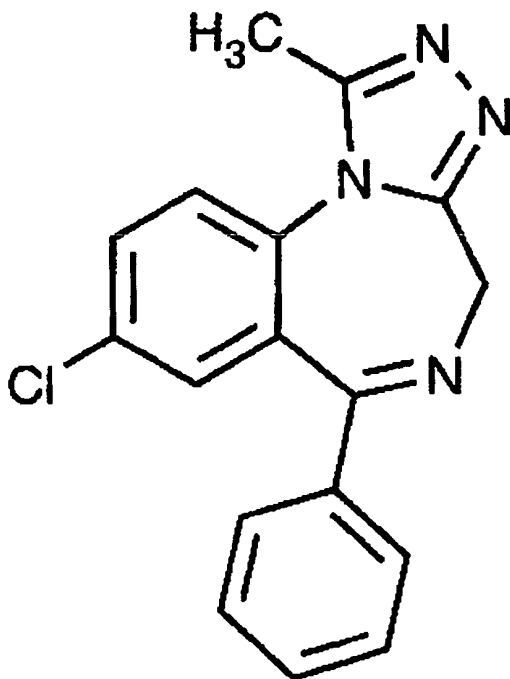
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ALPRAZALOM AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to alprazolam, as well as methods for protecting and administering alprazolam. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its effectiveness.

BACKGROUND OF THE INVENTION

Alprazolam is a known pharmaceutical agent that is used in the treatment of anxiety disorders. It is both commercially available and readily manufactured using
15 published synthetic schemes by those of ordinary skill in the art. Its structure is:



CW018P

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release

CW018P

through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide

CW018P

linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (alprazolam) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching alprazolam to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising alprazolam microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and alprazolam covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,

- (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Alprazolom preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting alprazolom from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering alprazolom to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the

polypeptide. In a preferred embodiment, alprazolam is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, alprazolam is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a

5 microencapsulating agent and alprazolam is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, alprazolam is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, alprazolam is released from the composition in a sustained release. In yet another preferred embodiment, the composition further

10 comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a

15 polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching alprazolam to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
- 20 from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second

25 agent, alprazolam and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an

30 amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side

CW018P

chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

- 5 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

10 DETAILED DESCRIPTION OF INVENTION

- The present invention provides several benefits for active agent delivery. First, the invention can stabilize alprazolam and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of alprazolam. Furthermore, active agents can be combined to produce synergistic effects. Also,
15 absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

- In the present invention, alprazolam is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
20 short chain of either amino acids or carbohydrates.

- The composition of the invention comprises alprazolam covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a
25 heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the

local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
5 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino
10 acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van
15 der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant
20 force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of
25 maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the

CW018P

amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to

poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

5 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
10 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
15 carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
20 specific properties to the drug delivery system.

 The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
25 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
30 alimentary tract can affect release.

CW018P

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
5 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, alprazolam is covalently attached to the polypeptide via the zzzzzzzz.

The polypeptide carrier can be prepared using conventional techniques. A
10 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG)
15 and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
20 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the
25 transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the

mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-alprazolam conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

CW018P

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
5 added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
10 solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

15 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

20 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product
25 precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product,
5 which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and
10 filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 alprazolom covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein alprazolom is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW018P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein alprazolam is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing alprazolam from said composition in a pH-dependent manner.

15 19. A method for protecting alprazolam from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of alprazolam from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching alprazolam to said polypeptide.

20 21. A method for delivering alprazolam to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
alprazolam covalently attached to said polypeptide.

25 22. The method of claim 21 wherein alprazolam is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein alprazolam is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

A composition comprising a polypeptide and alprazolam covalently attached to the polypeptide. Also provided is a method for delivery of alprazolam to a patient comprising administering to the patient a composition comprising a polypeptide and alprazolam covalently attached to the polypeptide. Also provided is a method for
15 protecting alprazolam from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of alprazolam from a composition comprising covalently attaching it to the polypeptide.

CW019P

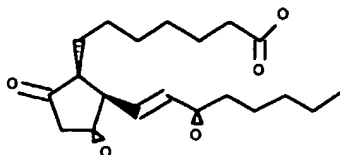
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ALPROSTADIL
AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to alprostadil, as well as methods for protecting and administering alprostadil. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Alprostadil is a known pharmaceutical agent that is used in the treatment of male
15 erectile dysfunction. Its chemical name is (11 α ,13E,15S)-11,15-dihydroxy-9-oxoprost-13-en-1-oic acid. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

CW019P

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

CW019P

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (alprostadil) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching alprostadil to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising alprostadil microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and alprostadil covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Alprostadil preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting alprostadil from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering alprostadil to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the
20 polypeptide. In a preferred embodiment, alprostadil is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, alprostadil is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and alprostadil is released from the composition by dissolution
25 of the microencapsulating agent. In another preferred embodiment, alprostadil is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, alprostadil is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

- (a) attaching alprostadil to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, alprostadil and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize alprostadil and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of alprostadil.

- 5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Alprostadil is the subject of U.S. Patent Number 5,741,523, herein incorporated by reference, which describes how to make that drug.

- 10 The composition of the invention comprises alprostadil covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

- Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW019P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

CW019P

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

25

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW019P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, alprostadil is covalently attached to the polypeptide via the carboxylic acid.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-alprostadil conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW019P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW019P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 alprostadil covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein alprostadil is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW019P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein alprostadil is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing alprostadil from said composition in a pH-dependent manner.

15 19. A method for protecting alprostadil from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of alprostadil from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching alprostadil to said polypeptide.

20 21. A method for delivering alprostadil to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
alprostadil covalently attached to said polypeptide.

25 22. The method of claim 21 wherein alprostadil is released from said composition by an enzyme-catalyzed release.

CW019P

23. The method of claim 21 wherein alprostadil is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and alprostadil covalently attached to the polypeptide. Also provided is a method for delivery of alprostadil to a patient comprising administering to the patient a composition comprising a polypeptide and alprostadil covalently attached to the polypeptide. Also provided is a method for protecting alprostadil from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of alprostadil from a composition comprising covalently attaching it to the polypeptide.

5

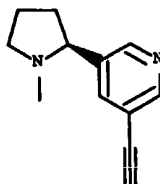
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ALTINICLINE
AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to altinicline, as well as methods for protecting and administering altinicline. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Altinicline is a known pharmaceutical agent that is used in the treatment of Parkinson's disease. Its chemical name is 3-ethynyl-5-[(2S)-1-methyl-2-pyrrolidinyl]pyridine. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW020P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some
5 technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically
10 require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the
15 gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The
20 released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that
25 incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly
30 or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW020P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (altinicline) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching altinicline to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising altinicline microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and altinicline covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Altinicline preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting altinicline from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering altinicline to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, altinicline is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, altinicline is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and altinicline is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, altinicline is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, altinicline is released from the composition in a sustained release.

CW020P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching altinicine to a side chain of an amino acid to form an active agent/amino acid complex;

10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, altinicine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW020P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize altinicline and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of altinicline. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Altinicline is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art.

 The composition of the invention comprises altinicline covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW020P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW020P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW020P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW020P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, altinicline is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
25 short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-altinicline conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 altinicine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein altinicine is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein altinicline is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing altinicline from said composition in a pH-dependent manner.

15 19. A method for protecting altinicline from degradation comprising covalently attaching said active agent to a polypeptide.

20 20. A method for controlling release of altinicline from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching altinicline to said polypeptide.

20 21. A method for delivering altinicline to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
altinicline covalently attached to said polypeptide.

25 22. The method of claim 21 wherein altinicline is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein altinicine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and altinicine covalently attached to the polypeptide. Also provided is a method for delivery of altinicine to a patient comprising administering to the patient a composition comprising a polypeptide and altinicine covalently attached to the polypeptide. Also provided is a method for protecting altinicine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of altinicine from a composition comprising covalently attaching it to the polypeptide.

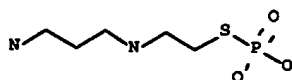
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING AMIFOSTINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to amifostine, as well as methods for protecting and administering amifostine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Amifostine is a known pharmaceutical agent that is used in the treatment of
15 moderate to severe xerostomia in patients with head and neck cancer undergoing postoperative radiotherapy. Its chemical name is 2-[(3-aminopropyl)amino]ethanethiol dihydrogen phosphate. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

CW021P

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of
10 cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

CW021P

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (amifostine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching amifostine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

 Alternatively, the present invention provides a pharmaceutical composition comprising amifostine microencapsulated by a polypeptide.

 The invention provides a composition comprising a polypeptide and amifostine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Amifostine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting amifostine from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering amifostine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the
20 polypeptide. In a preferred embodiment, amifostine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, amifostine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and amifostine is released from the composition by dissolution
25 of the microencapsulating agent. In another preferred embodiment, amifostine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, amifostine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

- (a) attaching amifostine to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, amifostine and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize amifostine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of amifostine.

- 5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Amifostine is the subject of U.S. Patent Numbers 5,424,471, 5,591,731, and 5,994,409, herein incorporated by reference, which describes how to make that drug.

- 10 The composition of the invention comprises amifostine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

- Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

CW021P

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW021P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, amifostine is covalently attached to the polypeptide via the phosphate group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

CW021P

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-amifostine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW021P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 amifostine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein amifostine is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein amifostine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing amifostine from said composition in a pH-dependent manner.

15 19. A method for protecting amifostine from degradation comprising covalently attaching said active agent to a polypeptide.

20 20. A method for controlling release of amifostine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching amifostine to said polypeptide.

20 21. A method for delivering amifostine to a patient comprising administering to said patient a composition comprising:
 a polypeptide; and
 amifostine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein amifostine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein amifostine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and amifostine covalently attached to the polypeptide. Also provided is a method for delivery of amifostine to a patient comprising administering to the patient a composition comprising a polypeptide and amifostine
5 covalently attached to the polypeptide. Also provided is a method for protecting amifostine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of amifostine from a composition comprising covalently attaching it to the polypeptide.

CW022P

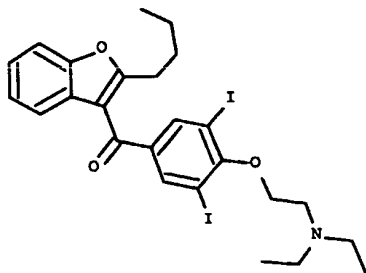
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING AMIODARONE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to amiodarone, as well as methods for protecting and administering amiodarone. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Amiodarone is a known pharmaceutical agent that is used in the treatment of
15 cardiac arrhythmia. Its chemical name is (2-butyl-3-benzofuranyl)[4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]methanone. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

CW022P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent
10 (amiodarone) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching amiodarone to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising amiodarone microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and amiodarone covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Amiodarone preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting amiodarone from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering amiodarone to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, amiodarone is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, amiodarone is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and amiodarone is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment,

CW022P

amiodarone is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, amiodarone is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and
5 release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
10 comprises the steps of:

- (a) attaching amiodarone to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
15 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, amiodarone and a second active agent can be copolymerized in step (c). In another
20 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
25 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize amiodarone and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of amiodarone. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises amiodarone covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW022P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
30

CW022P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, amiodarone is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
25 short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-amiodarone conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW022P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 amiodarone covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein amiodarone is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein amiodarone is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing amiodarone from said composition in a pH-dependent manner.

15 19. A method for protecting amiodarone from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of amiodarone from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching amiodarone to said polypeptide.

20 21. A method for delivering amiodarone to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
amiodarone covalently attached to said polypeptide.

25 22. The method of claim 21 wherein amiodarone is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein amiodarone is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and amiodarone covalently attached to the polypeptide. Also provided is a method for delivery of amiodarone to a patient comprising administering to the patient a composition comprising a polypeptide and
5 amiodarone covalently attached to the polypeptide. Also provided is a method for protecting amiodarone from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of amiodarone from a composition comprising covalently attaching it to the polypeptide.

CW023P

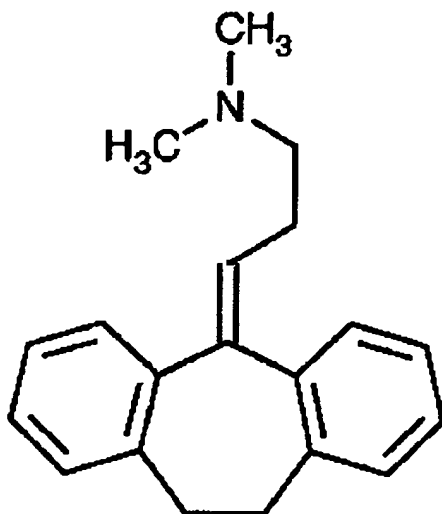
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING AMITRIPTYLINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to amitriptyline, as well as methods for protecting and administering amitriptyline. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Amitriptyline is a known pharmaceutical agent that is used in the treatment of depression. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability

of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified

CW023P

amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that

CW023P

incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable
5 diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR
10 application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (amitriptyline) to a polymer of peptides or amino acids. The invention is distinguished
15 from the above-mentioned technologies by virtue of covalently attaching amitriptyline to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is
20 controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition
25 comprising amitriptyline microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and amitriptyline covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a

CW023P

heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

5 Amitriptyline preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is
10 an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The
15 microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be
20 conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

 The invention also provides a method for protecting amitriptyline from degradation comprising covalently attaching it to a polypeptide.

25 The invention also provides a method for delivering amitriptyline to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, amitriptyline is released from the composition

CW023P

by an enzyme-catalyzed release. In another preferred embodiment, amitriptyline is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and amitriptyline is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, amitriptyline is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, amitriptyline is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching amitriptyline to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, amitriptyline and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a

CW023P

carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize amitriptyline and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of amitriptyline. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises amitriptyline covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW023P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW023P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW023P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW023P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW023P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, amitriptyline is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

CW023P

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

5 The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex,
10 PEG or salts.

 There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
15 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
20 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

 In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
25 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

CW023P

Preferably, the resultant peptide-amitriptyline conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

5 **Acid/N-terminus conjugation**

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product
10 precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
15 followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene
20 produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified
25 using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated

CW023P

solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

5 Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered,
10 dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for
15 several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes
20 homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically
25 overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

CW023P

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW023P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 amitriptyline covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein amitriptyline is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein amitriptyline is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing amitriptyline from said composition in a pH-dependent manner.

15 19. A method for protecting amitriptyline from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of amitriptyline from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching amitriptyline to said polypeptide.

20 21. A method for delivering amitriptyline to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
amitriptyline covalently attached to said polypeptide.

25 22. The method of claim 21 wherein amitriptyline is released from said composition by an enzyme-catalyzed release.

CW023P

23. The method of claim 21 wherein amitriptyline is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

- 10 A composition comprising a polypeptide and amitriptyline covalently attached to the polypeptide. Also provided is a method for delivery of amitriptyline to a patient comprising administering to the patient a composition comprising a polypeptide and amitriptyline covalently attached to the polypeptide. Also provided is a method for protecting amitriptyline from degradation comprising covalently attaching it to a
- 15 polypeptide. Also provided is a method for controlling release of amitriptyline from a composition comprising covalently attaching it to the polypeptide.

CW024P

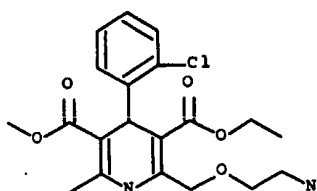
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING AMLODIPINE BESYLATE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to amlodipine besylate, as well as methods for protecting and administering amlodipine besylate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Amlodipine besylate is a known pharmaceutical agent that is used in the
15 treatment and prevention of myocardial infarction and stroke. Its chemical name is 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid, 3-ethyl 5-methyl ester monobenzenesulfonate. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

CW024P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW024P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some
5 technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically
10 require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the
15 gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The
20 released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that
25 incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly
30 or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW024P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (amlodipine besylate) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching amlodipine besylate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising amlodipine besylate microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and amlodipine besylate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Amlodipine besylate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active

CW024P

agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet
5 another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino
10 acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In
15 another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting amlodipine besylate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering amlodipine besylate to a
20 patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, amlodipine besylate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, amlodipine besylate is released in a time-dependent manner based on the
25 pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and amlodipine besylate is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, amlodipine besylate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, amlodipine

CW024P

besylate is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching amlodipine besylate to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, amlodipine besylate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW024P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize amlodipine besylate and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of amlodipine besylate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Amlodipine besylate is the subject of U.S. Patent Numbers 4,572,909 and 4,879,303, herein incorporated by reference, which describe how to make that drug.

15 The composition of the invention comprises amlodipine besylate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW024P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

- 5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
- 10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
- 15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

- Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
- 20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
- 25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW024P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW024P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW024P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW024P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, amlodipine besylate is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW024P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-amlodipine besylate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW024P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 amlodipine besylate covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein amlodipine besylate is covalently
attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein amlodipine besylate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing amlodipine besylate from said composition in a pH-dependent manner.

15 19. A method for protecting amlodipine besylate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of amlodipine besylate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching amlodipine besylate to said polypeptide.

20 21. A method for delivering amlodipine besylate to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
amlodipine besylate covalently attached to said polypeptide.

25 22. The method of claim 21 wherein amlodipine besylate is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein amlodipine besylate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and amlodipine besylate covalently attached to the polypeptide. Also provided is a method for delivery of amlodipine besylate to a patient comprising administering to the patient a composition comprising a polypeptide and amlodipine besylate covalently attached to the polypeptide. Also provided is a method for protecting amlodipine besylate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of amlodipine besylate from a composition comprising covalently attaching it to the polypeptide.

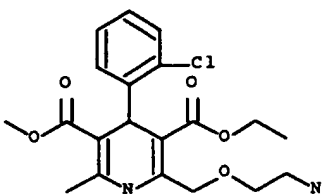
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING AMLODIPINE
BESYLATE AND BENAZEPRIL AND METHODS OF
MAKING AND USING SAME**

5 FIELD OF THE INVENTION

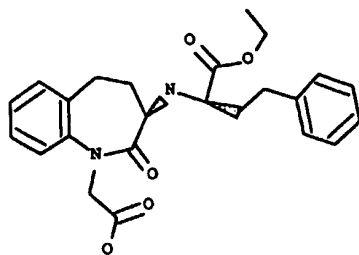
The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to amlodipine besylate, as well as methods for protecting and administering amlodipine besylate and benazepril. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Amlodipine besylate is a known pharmaceutical agent that is used in the treatment and prevention of myocardial infarction and stroke. Its chemical name is 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid, 3-ethyl 5-methyl ester monobenzenesulfonate. Its structure is:



20 Benazepril has the chemical name [S-(R*,R*)]-3-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-acetic acid. It is available commercially or can be made by those of skill in the art. Its structure is as follows:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where

the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (amlodipine besylate and benazepril) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching amlodipine besylate and benazepril to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively

hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising amlodipine besylate and benazepril microencapsulated by a polypeptide.

5 The invention provides a composition comprising a polypeptide and amlodipine besylate and benazepril covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more
10 synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Amlodipine besylate and benazepril preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the
15 polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

20 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

25 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In

another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting amlodipine besylate and benazepril from degradation comprising covalently attaching it to a polypeptide.

5 The invention also provides a method for delivering amlodipine besylate and benazepril to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, amlodipine besylate and benazepril are released from the composition by an enzyme-catalyzed release. In
10 another preferred embodiment, amlodipine besylate and benazepril are released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and amlodipine besylate and benazepril are released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment,
15 amlodipine besylate and benazepril are released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, amlodipine besylate and benazepril is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the
20 polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- 25 (a) attaching amlodipine besylate and benazepril to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, amlodipine besylate and benazepril and a second active agent can be
5 copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic
10 acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant
15 group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is
20 described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize amlodipine besylate and benazepril and prevent its digestion in
25 the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of amlodipine besylate and benazepril. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Amlodipine besylate is the subject of U.S. Patent Numbers 4,572,909 and 4,879,303, herein incorporated by reference, which describe how to make that drug.

The composition of the invention comprises amlodipine besylate and benazepril covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
5 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

10 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The
15 folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a
20 particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

25 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with

protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
5 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
10 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
15 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of
20 the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be
25 enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will

ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
5 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
10 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
15 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
20 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate
25 weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the

jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

5

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order

to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
5 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

10 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
15 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
20 any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
25 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
30 polypeptides through a spacer or linker on the pendant group, which is terminated,

preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
5 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
10 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, amlodipine besylate is covalently attached to the polypeptide via the amino group. Benazepril is attached via its carboxylic acid.

The polypeptide carrier can be prepared using conventional techniques. A
15 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG)
20 and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
25 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known

intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
5 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
10 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

15 Preferably, the resultant peptide-amlodipine besylate and benazepril conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

20 **Acid/N-terminus conjugation**

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product
25 precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product
5 precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product,
10 which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and
15 filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 amlodipine besylate and benazepril covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein amlodipine besylate and benazepril are
covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein amlodipine besylate and benazepril are conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing amlodipine besylate and benazepril from said composition in a pH-dependent manner.

15 19. A method for protecting amlodipine besylate and benazepril from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of amlodipine besylate and benazepril from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching amlodipine besylate and benazepril to said polypeptide.

20 21. A method for delivering amlodipine besylate and benazepril to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
amlodipine besylate and benazepril covalently attached to said polypeptide.

25 22. The method of claim 21 wherein amlodipine besylate and benazepril are released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein amlodipine besylate and benazepril is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

10 A composition comprising a polypeptide and amlodipine besylate and benazepril covalently attached to the polypeptide. Also provided is a method for delivery of amlodipine besylate and benazepril to a patient comprising administering to the patient a composition comprising a polypeptide and amlodipine besylate and benazepril covalently attached to the polypeptide. Also provided is a method for protecting amlodipine
15 besylate and benazepril from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of amlodipine besylate and benazepril from a composition comprising covalently attaching it to the polypeptide.

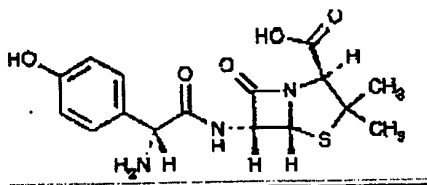
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING AMOXICILLIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to amoxicillin, as well as methods for protecting and administering amoxicillin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Amoxicillin is a known pharmaceutical agent that is used in the treatment of bacterial infection. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (amoxicillin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching amoxicillin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising amoxicillin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and amoxicillin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Amoxicillin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting amoxicillin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering amoxicillin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, amoxicillin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, amoxicillin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and amoxicillin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, amoxicillin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, amoxicillin is released from the composition in a

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching amoxicillin to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, amoxicillin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize amoxicillin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of amoxicillin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises amoxicillin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

CW026P

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, amoxicillin is covalently attached to the polypeptide via the carboxylic acid.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

CW026P

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-amoxicillin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 amoxicillin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein amoxicillin is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein amoxicillin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing amoxicillin from said composition in a pH-dependent manner.

15 19. A method for protecting amoxicillin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of amoxicillin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching amoxicillin to said polypeptide.

20 21. A method for delivering amoxicillin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
amoxicillin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein amoxicillin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein amoxicillin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and amoxicillin covalently attached to the polypeptide. Also provided is a method for delivery of amoxicillin to a patient comprising administering to the patient a composition comprising a polypeptide and amoxicillin covalently attached to the polypeptide. Also provided is a method for
5 protecting amoxicillin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of amoxicillin from a composition comprising covalently attaching it to the polypeptide.

CW027P

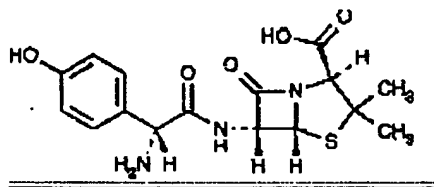
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
AMOXICILLIN AND CLAVULANATE AND METHODS OF MAKING AND
USING SAME**

5 **FIELD OF THE INVENTION**

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to amoxicillin and clavulanate, as well as methods for protecting and administering amoxicillin and clavulanate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Amoxicillin and clavulanate are known pharmaceutical agents used in the treatment of bacterial infections. Each is available commercially and can be made by those of ordinary skill in the art. The structure of amoxicillin is:



20 The structure of clavulanate is (Z)-(2R,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]-heptane-2-carboxylate.

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

CW027P

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW027P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

 It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW027P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent
10 (amoxicillin and clavulanate) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching amoxicillin and clavulanate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily
15 in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release
20 mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising amoxicillin and clavulanate microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and amoxicillin and clavulanate covalently attached to the polypeptide. Preferably, the polypeptide is (i)
25 an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Amoxicillin and clavulanate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting amoxicillin and clavulanate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering amoxicillin and clavulanate to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, amoxicillin and clavulanate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, amoxicillin and clavulanate is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and amoxicillin and clavulanate is released from the composition by dissolution of the

CW027P

microencapsulating agent. In another preferred embodiment, amoxicillin and clavulanate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, amoxicillin and clavulanate is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching amoxicillin and clavulanate to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, amoxicillin and clavulanate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000,
5 incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize amoxicillin and clavulanate and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of
10 amoxicillin and clavulanate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Amoxicillin and clavulanate is the subject of U.S. Patent Number yyyyy, herein
15 incorporated by reference, which describes how to make that drug.

The composition of the invention comprises amoxicillin and clavulanate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a
20 synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
25 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW027P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW027P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, amoxicillin and clavulanate are each covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW027P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

CW027P

Preferably, the resultant peptide-amoxicillin and clavulanate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids
5 and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be
10 stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
15 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

20 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
25 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-
5 hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic
10 acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
15 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

20 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

25 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically

overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
5 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 amoxicillin and clavulanate covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein amoxicillin and clavulanate is covalently
attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein amoxicillin and clavulanate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing amoxicillin and clavulanate from said composition in a pH-dependent manner.

15 19. A method for protecting amoxicillin and clavulanate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of amoxicillin and clavulanate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching amoxicillin and clavulanate to said polypeptide.

20 21. A method for delivering amoxicillin and clavulanate to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
amoxicillin and clavulanate covalently attached to said polypeptide.

25 22. The method of claim 21 wherein amoxicillin and clavulanate is released from said composition by an enzyme-catalyzed release.

CW027P

23. The method of claim 21 wherein amoxicillin and clavulanate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

10 A composition comprising a polypeptide and amoxicillin and clavulanate covalently attached to the polypeptide. Also provided is a method for delivery of amoxicillin and clavulanate to a patient comprising administering to the patient a composition comprising a polypeptide and amoxicillin and clavulanate covalently attached to the polypeptide. Also provided is a method for protecting amoxicillin and
15 clavulanate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of amoxicillin and clavulanate from a composition comprising covalently attaching it to the polypeptide.

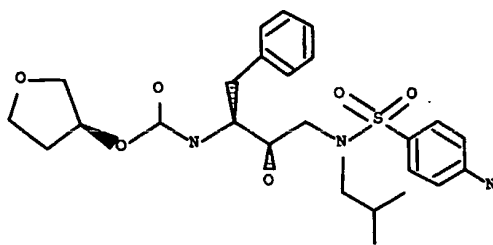
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING AMPRENAVIR AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to amprenavir, as well as methods for protecting and administering amprenavir. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Amprenavir, a proteinase inhibitor, is a known pharmaceutical agent that is used in the treatment of HIV infection. Its chemical name is [3S-3R*(1R*,2S*)]]-[3-[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic acid tetrahydro-3-furanyl ester. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.

20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

30

CW028P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent
10 (amprenavir) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching amprenavir to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising amprenavir microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and amprenavir covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Amprenavir preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting amprenavir from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering amprenavir to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, amprenavir is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, amprenavir is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and amprenavir is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, amprenavir is

released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, amprenavir is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant
5 from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
10 comprises the steps of:

- (a) attaching amprenavir to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
15 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, amprenavir and a second active agent can be copolymerized in step (c). In another
20 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
25 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000,
5 incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize amprenavir and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of amprenavir.
10 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Amprenavir is the subject of U.S. Patent Numbers 5,585,397, 5,646,180, and 5,723,490, herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises amprenavir covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
20 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
25 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, amprenavir is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW028P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-amprenavir conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

5 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 amprenavir covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein amprenavir is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein amprenavir is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing amprenavir from said composition in a pH-dependent manner.

15 19. A method for protecting amprenavir from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of amprenavir from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching amprenavir to said polypeptide.

20 21. A method for delivering amprenavir to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
amprenavir covalently attached to said polypeptide.

25 22. The method of claim 21 wherein amprenavir is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein amprenavir is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

10 A composition comprising a polypeptide and amprenavir covalently attached to the polypeptide. Also provided is a method for delivery of amprenavir to a patient comprising administering to the patient a composition comprising a polypeptide and amprenavir covalently attached to the polypeptide. Also provided is a method for protecting amprenavir from degradation comprising covalently attaching it to a
15 polypeptide. Also provided is a method for controlling release of amprenavir from a composition comprising covalently attaching it to the polypeptide.

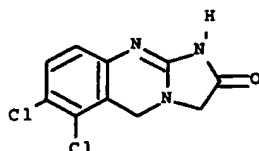
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ANAGRELIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to anagrelide, as well as methods for protecting and administering anagrelide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Anagrelide is a known pharmaceutical agent that is used as a platelet reducing drug. Its chemical name is 6,7-dichloro-1,5-dihydroimidazo[2,1-b]quinazolin-2(3H)-one. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

CW029P

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (anagrelide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching anagrelide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

 Alternatively, the present invention provides a pharmaceutical composition comprising anagrelide microencapsulated by a polypeptide.

 The invention provides a composition comprising a polypeptide and anagrelide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Anagrelide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting anagrelide from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering anagrelide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the
20 polypeptide. In a preferred embodiment, anagrelide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, anagrelide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and anagrelide is released from the composition by dissolution
25 of the microencapsulating agent. In another preferred embodiment, anagrelide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, anagrelide is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

CW029P

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

(a) attaching anagrelide to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, anagrelide and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize anagrelide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of anagrelide.

5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Anagrelide is the subject of GB patent 1418822 (1975), (based on U.S. Patent Application Serial Number 223,723, filed in 1972, herein incorporated by reference,
10 which describes how to make that drug.

The composition of the invention comprises anagrelide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a
15 heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and
20 turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on
25 the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the

CW029P

protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of
15 maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the
20 amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.
25 Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

20

CW029P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW029P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, anagrelide is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-anagrelide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 anagrelide covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein anagrelide is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein anagrelide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing anagrelide from said composition in a pH-dependent manner.

15 19. A method for protecting anagrelide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of anagrelide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching anagrelide to said polypeptide.

20 21. A method for delivering anagrelide to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
anagrelide covalently attached to said polypeptide.

25 22. The method of claim 21 wherein anagrelide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein anagrelide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and anagrelide covalently attached to the polypeptide. Also provided is a method for delivery of anagrelide to a patient comprising administering to the patient a composition comprising a polypeptide and anagrelide covalently attached to the polypeptide. Also provided is a method for protecting anagrelide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of anagrelide from a composition comprising covalently attaching it to the polypeptide.

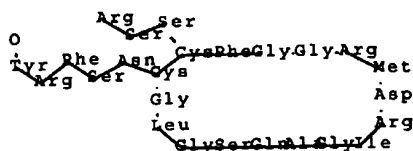
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ANARITIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to anaritide, as well as methods for protecting and administering anaritide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Anaritide is a known pharmaceutical agent that is used in the treatment of oliguric acute renal failure. Its chemical name is N-L-arginyl-8-L-methionine-21a-L-phenylalanine-21b-L-arginine-21c-L-tyrosine-atRIOpeptin-21. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

CW030P

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

CW030P

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (anaritide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching anaritide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will
10 stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a
15 second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising anaritide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and anaritide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Anaritide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting anaritide from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering anaritide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, anaritide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, anaritide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and anaritide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, anaritide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, anaritide is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

20

25

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

(a) attaching anaritide to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, anaritide and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize anaritide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of anaritide. Furthermore, 5 active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises anaritide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one 10 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have 15 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains 20 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino 25 acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

CW030P

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

CW030P

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
 5 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
 15 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
 20 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, anaritide is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

CW030P

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-anaritide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- 20 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 anarotide covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein anarotide is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein anaritide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing anaritide from said composition in a pH-dependent manner.

15 19. A method for protecting anaritide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of anaritide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching anaritide to said polypeptide.

20 21. A method for delivering anaritide to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
anaritide covalently attached to said polypeptide.

25 22. The method of claim 21 wherein anaritide is released from said composition by an enzyme-catalyzed release.

CW030P

23. The method of claim 21 wherein anarotide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and anaritide covalently attached to the polypeptide. Also provided is a method for delivery of anaritide to a patient comprising administering to the patient a composition comprising a polypeptide and anaritide covalently attached to the polypeptide. Also provided is a method for protecting anaritide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of anaritide from a composition comprising covalently attaching it to the polypeptide.

CW031P

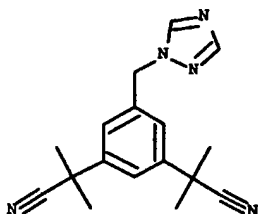
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ANASTROZOLE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to anastrozole, as well as methods for protecting and administering anastrozole. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Anastrozole is a known pharmaceutical agent that is used in the treatment of
15 breast cancer. Its chemical name is alpha,alpha',alpha',alpha'-tetramethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-benzenediacetonitrile. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW031P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW031P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (anastrozole) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching anastrozole to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising anastrozole microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and anastrozole covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Anastrozole preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW031P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting anastrozole from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering anastrozole to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, anastrozole is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, anastrozole is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and anastrozole is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, anastrozole is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, anastrozole is released from the composition in a

CW031P

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching anastrozole to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, anastrozole and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is

CW031P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize anastrozole and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of anastrozole. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Anastrozole is the subject of EP 296749 B (1994), priority GB 14013 (1987), herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises anastrozole covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW031P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

- 5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
- 10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
- 15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

- Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
- 20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
- 25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW031P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
25 short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW031P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-anastrozole conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW031P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW031P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 anastrozole covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein anastrozole is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein anastrozole is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing anastrozole from said composition in a pH-dependent manner.

15 19. A method for protecting anastrozole from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of anastrozole from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching anastrozole to said polypeptide.

20 21. A method for delivering anastrozole to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

anastrozole covalently attached to said polypeptide.

25 22. The method of claim 21 wherein anastrozole is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein anastrozole is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and anastrozole covalently attached to the polypeptide. Also provided is a method for delivery of anastrozole to a patient comprising administering to the patient a composition comprising a polypeptide and anastrozole covalently attached to the polypeptide. Also provided is a method for
5 protecting anastrozole from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of anastrozole from a composition comprising covalently attaching it to the polypeptide.

**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
ANTISENSE OLIGONUCLEOTIDES AND METHODS OF MAKING AND
USING SAME**

5 FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to antisense oligonucleotides, as well as methods for protecting and administering antisense oligonucleotides. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Antisense oligonucleotides are a class of compounds made of RNA that is complementary to the mRNA that produces a protein of interest. Their usefulness is primarily for gene therapy. Individual uses include those for the treatment of inflammatory bowel diseases (). They are made by chemical RNA synthesis or, alternatively, by using a gene construct containing the antisense orientation of the gene of interest and isolating the RNA of interest.

20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (antisense oligonucleotides) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching antisense oligonucleotides to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In
10 certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier
15 peptide. This enzymatic action introduces a second order sustained release mechanism.

 Alternatively, the present invention provides a pharmaceutical composition comprising antisense oligonucleotides microencapsulated by a polypeptide.

 The invention provides a composition comprising a polypeptide and antisense oligonucleotides covalently attached to the polypeptide. Preferably, the polypeptide is (i)
20 an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Antisense oligonucleotides preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached

CW032P

to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting antisense oligonucleotides from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering antisense oligonucleotides to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently
20 attached to the polypeptide. In a preferred embodiment, antisense oligonucleotides is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, antisense oligonucleotides is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and antisense
25 oligonucleotides is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, antisense oligonucleotides is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, antisense oligonucleotides is released from the composition in a sustained release. In yet another preferred embodiment, the

composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching antisense oligonucleotides to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, antisense oligonucleotides and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident
20 intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred
25 embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize antisense oligonucleotides and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of antisense oligonucleotides. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises antisense oligonucleotides covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the

CW032P

protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW032P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW032P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW032P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW032P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, antisense oligonucleotides are covalently attached to the polypeptide via the ribose hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW032P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-antisense oligonucleotides conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW032P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW032P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 antisense oligonucleotides covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein antisense oligonucleotides is covalently
attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW032P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein antisense oligonucleotides is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing antisense oligonucleotides from said composition in a pH-dependent manner.

15 19. A method for protecting antisense oligonucleotides from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of antisense oligonucleotides from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching antisense oligonucleotides to said polypeptide.

20 21. A method for delivering antisense oligonucleotides to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
antisense oligonucleotides covalently attached to said polypeptide.

25 22. The method of claim 21 wherein antisense oligonucleotides is released from said composition by an enzyme-catalyzed release.

CW032P

23. The method of claim 21 wherein antisense oligonucleotides is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

10 A composition comprising a polypeptide and antisense oligonucleotides covalently attached to the polypeptide. Also provided is a method for delivery of antisense oligonucleotides to a patient comprising administering to the patient a composition comprising a polypeptide and antisense oligonucleotides covalently attached to the polypeptide. Also provided is a method for protecting antisense oligonucleotides
15 from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of antisense oligonucleotides from a composition comprising covalently attaching it to the polypeptide.

CW033P

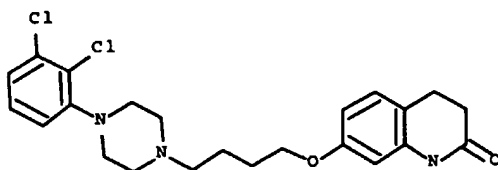
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ARIPIRAZOLE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to aripiprazole, as well as methods for protecting and administering aripiprazole. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Aripiprazole is a known pharmaceutical agent that is used in reducing both the
15 positive and negative symptoms of acutely psychotic patients. Its chemical name is 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydro-2(1H)-quinolinone. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

CW033P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW033P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW033P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (aripiprazole) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching aripiprazole to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising aripiprazole microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and aripiprazole covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Aripiprazole preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW033P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting aripiprazole from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering aripiprazole to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, aripiprazole is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, aripiprazole is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and aripiprazole is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, aripiprazole is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, aripiprazole is released from the

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching aripiprazole to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
(NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, aripiprazole and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

CW033P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize aripiprazole and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of aripiprazole. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Aripiprazole is the subject of EP 367141 B (1996)(priority Japan 276953 (1988)), herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises aripiprazole covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW033P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW033P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW033P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-aripiprazole conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW033P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 aripiprazole covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein aripiprazole is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW033P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein aripiprazole is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing aripiprazole from said composition in a pH-dependent manner.

15 19. A method for protecting aripiprazole from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of aripiprazole from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching aripiprazole to said polypeptide.

20 21. A method for delivering aripiprazole to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
aripiprazole covalently attached to said polypeptide.

25 22. The method of claim 21 wherein aripiprazole is released from said composition by an enzyme-catalyzed release.

Abstract

A composition comprising a polypeptide and aripiprazole covalently attached to the polypeptide. Also provided is a method for delivery of aripiprazole to a patient comprising administering to the patient a composition comprising a polypeptide and aripiprazole covalently attached to the polypeptide. Also provided is a method for protecting aripiprazole from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of aripiprazole from a composition comprising covalently attaching it to the polypeptide.

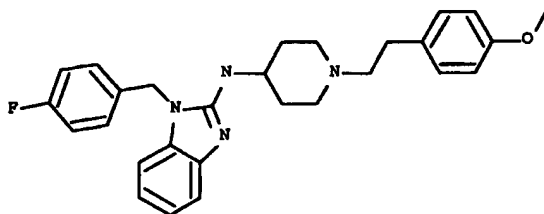
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ASTEMIZOLE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to astemizole, as well as methods for protecting and administering astemizole. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Astemizole is a known pharmaceutical agent that is used in the treatment of
15 seasonal allergic rhinitis and chronic idiopathic urticaria. Its chemical name is 1-[(4-fluorophenyl)methyl]-N-[1-[2-(4-methoxyphenyl)ethyl]-4-piperidinyl]-1H-benzimidazol-2-amine. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble
5 microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent
10 (astemizole) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching astemizole to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising astemizole microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and astemizole covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Astemizole preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting astemizole from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering astemizole to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, astemizole is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, astemizole is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and astemizole is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, astemizole is

released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, astemizole is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated
5 into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- 10 (a) attaching astemizole to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
15 (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, astemizole and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released
20 from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side
25 chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the
30 following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize astemizole and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of astemizole. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also
10 allows targeted delivery of active agents to specific sites of action.

 The composition of the invention comprises astemizole covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a
15 heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and
20 turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on
25 the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the

protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of
15 maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the
20 amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.
25 Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

20

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
30

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
25 short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-astemizole conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 astemizole covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein astemizole is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein astemizole is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing astemizole from said composition in a pH-dependent manner.

15 19. A method for protecting astemizole from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of astemizole from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching astemizole to said polypeptide.

20 21. A method for delivering astemizole to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
astemizole covalently attached to said polypeptide.

25 22. The method of claim 21 wherein astemizole is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein astemizole is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and astemizole covalently attached to the polypeptide. Also provided is a method for delivery of astemizole to a patient comprising administering to the patient a composition comprising a polypeptide and
5 astemizole covalently attached to the polypeptide. Also provided is a method for protecting astemizole from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of astemizole from a composition comprising covalently attaching it to the polypeptide.

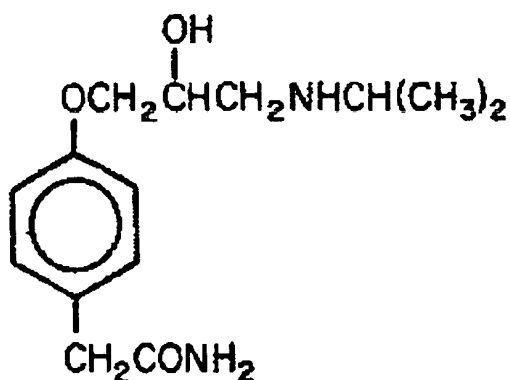
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ATENOLOL
AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to atenolol, as well as methods for protecting and administering atenolol. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Atenolol is a known pharmaceutical agent that is used in the treatment of
15 hypertension or chronic stable angina pectoris in patients with chronic obstructive pulmonary disease (COPD) or type 1 diabetes mellitus. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered

product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

- 5 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another
- 10 invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

- Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of
- 15 cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme
- 20 degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

- Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example,
- 25 copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (atenolol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching atenolol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising atenolol microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and atenolol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Atenolol preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a
5 carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-
10 terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
15 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
20 composition in a pH-dependent manner.

The invention also provides a method for protecting atenolol from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering atenolol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a
25 composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, atenolol is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, atenolol is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release.

In another preferred embodiment, the composition further comprises a microencapsulating agent and atenolol is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, atenolol is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, atenolol is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

10 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching atenolol to a side chain of an amino acid to form an active agent/amino acid complex;

15 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, atenolol and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

20
25
30

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000,
5 incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize atenolol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of atenolol. Furthermore,
10 active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises atenolol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
15 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have
20 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyridoxine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, atenolol is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-atenolol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 atenolol covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein atenolol is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein atenolol is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing atenolol from said composition in a pH-dependent manner.

15 19. A method for protecting atenolol from degradation comprising covalently attaching said active agent to a polypeptide.

20 20. A method for controlling release of atenolol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching atenolol to said polypeptide.

21. A method for delivering atenolol to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
atenolol covalently attached to said polypeptide.

22. The method of claim 21 wherein atenolol is released from said composition
25 by an enzyme-catalyzed release.

23. The method of claim 21 wherein atenolol is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and atenolol covalently attached to the polypeptide. Also provided is a method for delivery of atenolol to a patient comprising administering to the patient a composition comprising a polypeptide and atenolol covalently attached to the polypeptide. Also provided is a method for protecting atenolol from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of atenolol from a composition comprising covalently attaching it to the polypeptide.

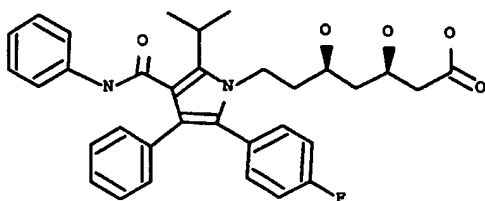
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ATORVASTATIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to atorvastatin, as well as methods for protecting and administering atorvastatin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Atorvastatin is a known pharmaceutical agent that is used in the treatment of high
15 cholesterol. Its chemical name is (betaR,deltaR)-2-(4-fluorophenyl)-beta,delta-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (atorvastatin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching atorvastatin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising atorvastatin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and atorvastatin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Atorvastatin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting atorvastatin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering atorvastatin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, atorvastatin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, atorvastatin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and atorvastatin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, atorvastatin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, atorvastatin is released from the

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching atorvastatin to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, atorvastatin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize atorvastatin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of atorvastatin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Atorvastatin is the subject of U.S. Patent Numbers 4,681,893, 5,273,995, 5,686,104, and 5,969,156, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises atorvastatin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior
10 and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, atorvastatin is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-atorvastatin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 atorvastatin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein atorvastatin is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein atorvastatin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing atorvastatin from said composition in a pH-dependent manner.

15 19. A method for protecting atorvastatin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of atorvastatin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching atorvastatin to said polypeptide.

20 21. A method for delivering atorvastatin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
atorvastatin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein atorvastatin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein atorvastatin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and atorvastatin covalently attached to the polypeptide. Also provided is a method for delivery of atorvastatin to a patient comprising administering to the patient a composition comprising a polypeptide and
5 atorvastatin covalently attached to the polypeptide. Also provided is a method for protecting atorvastatin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of atorvastatin from a composition comprising covalently attaching it to the polypeptide.

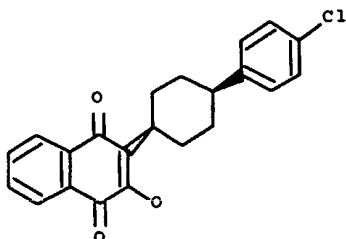
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ATOVAQUONE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to atovaquone, as well as methods for protecting and administering atovaquone. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Atovaquone is a known pharmaceutical agent that is used in the prevention of
15 *Pneumocystis carinii* pneumonia. Its chemical name is 2-[trans-4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthalenedione. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (atovaquone) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching atovaquone to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising atovaquone microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and atovaquone covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Atovaquone preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting atovaquone from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering atovaquone to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, atovaquone is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, atovaquone is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and atovaquone is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, atovaquone is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, atovaquone is released from the composition in a

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching atovaquone to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, atovaquone and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW037P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize atovaquone and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of atovaquone. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Atovaquone is the subject of U.S. Patent Numbers 4,981,874 and 5,053,432, herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises atovaquone covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW037P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW037P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW037P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW037P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, atovaquone is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW037P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-atovaquone conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW037P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 atovaquone covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein atovaquone is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein atovaquone is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing atovaquone from said composition in a pH-dependent manner.

15 19. A method for protecting atovaquone from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of atovaquone from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching atovaquone to said polypeptide.

20 21. A method for delivering atovaquone to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
atovaquone covalently attached to said polypeptide.

25 22. The method of claim 21 wherein atovaquone is released from said composition by an enzyme-catalyzed release.

CW037P

23. The method of claim 21 wherein atovaquone is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and atovaquone covalently attached to the polypeptide. Also provided is a method for delivery of atovaquone to a patient comprising administering to the patient a composition comprising a polypeptide and atovaquone covalently attached to the polypeptide. Also provided is a method for
5 protecting atovaquone from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of atovaquone from a composition comprising covalently attaching it to the polypeptide.

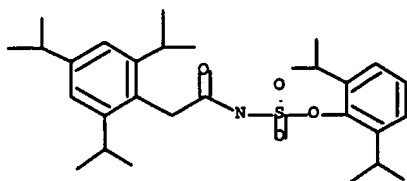
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING AVASIMIBE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to avasimibe, as well as methods for protecting and administering avasimibe. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Avasimibe is a known pharmaceutical agent that is used in the treatment of
15 hyperlipidemia. Its chemical name is N-[[2,6-bis(1-methylethyl)phenoxy]sulfonyl]-2,4,6-tris(1-methylethyl)benzeneacetamide. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered
product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical
compound may contain one or more of the following: another active pharmaceutical
agent, an adjuvant, or an inhibitor.

CW038P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW038P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW038P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (avasimibe) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching avasimibe to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising avasimibe microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and avasimibe covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Avasimibe preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW038P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting avasimibe from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering avasimibe to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, avasimibe is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, avasimibe is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and avasimibe is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, avasimibe is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, avasimibe is released from the composition in a sustained release.

CW038P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching avasimibe to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, avasimibe and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW038P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize avasimibe and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of avasimibe. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Avasimibe is the subject of WO 94/26702 1994 (priority US 62515 (1993)), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises avasimibe covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW038P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW038P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border

10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For

20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW038P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
30

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, avasimibe is covalently attached to the polypeptide via the sulfate group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-avasimibe conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW038P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW038P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 avasimibe covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein avasimibe is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW038P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein avasimibe is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing avasimibe from said composition in a pH-dependent manner.

15 19. A method for protecting avasimibe from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of avasimibe from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching avasimibe to said polypeptide.

20 21. A method for delivering avasimibe to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
avasimibe covalently attached to said polypeptide.

25 22. The method of claim 21 wherein avasimibe is released from said composition by an enzyme-catalyzed release.

CW038P

23. The method of claim 21 wherein avasimibe is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW038P

Abstract

A composition comprising a polypeptide and avasimibe covalently attached to the polypeptide. Also provided is a method for delivery of avasimibe to a patient comprising administering to the patient a composition comprising a polypeptide and avasimibe covalently attached to the polypeptide. Also provided is a method for protecting avasimibe from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of avasimibe from a composition comprising covalently attaching it to the polypeptide.

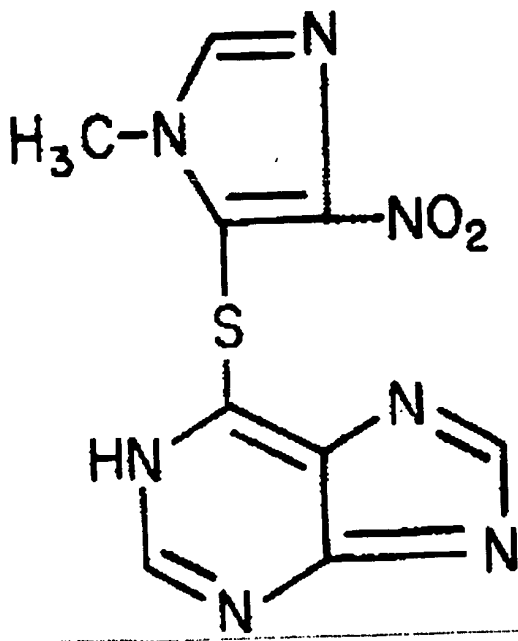
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
AZATHIOPRENE AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to azathioprene, as well as methods for protecting and administering azathioprene. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Azathioprene is a known pharmaceutical agent that is used in the treatment of transplant organ rejection. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



CW039P

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release

CW039P

through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide

CW039P

linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

- It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.
- Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent (azathioprene) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching azathioprene to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- Alternatively, the present invention provides a pharmaceutical composition comprising azathioprene microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and azathioprene covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,

CW039P

(ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Azathioprene preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting azathioprene from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering azathioprene to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the

CW039P

polypeptide. In a preferred embodiment, azathioprene is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, azathioprene is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a
5 microencapsulating agent and azathioprene is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, azathioprene is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, azathioprene is released from the composition in a sustained release. In yet another preferred embodiment, the
10 composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a
15 polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching azathioprene to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
20 from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second
25 agent, azathioprene and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an
30 amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side

CW039P

chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

5 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

10 DETAILED DESCRIPTION OF INVENTION

 The present invention provides several benefits for active agent delivery. First, the invention can stabilize azathioprene and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of azathioprene. Furthermore, active agents can be combined to produce synergistic effects. Also,
15 absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

 The composition of the invention comprises azathioprene covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
20 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
25 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW039P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW039P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW039P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW039P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW039P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, azathioprene is covalently attached to the polypeptide via the nitrate group, or alternatively through a linker.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW039P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-azathioprene conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW039P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW039P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 azathioprene covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein azathioprene is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein azathioprene is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing azathioprene from said composition in a pH-dependent manner.

15 19. A method for protecting azathioprene from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of azathioprene from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching azathioprene to said polypeptide.

20 21. A method for delivering azathioprene to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
azathioprene covalently attached to said polypeptide.

25 22. The method of claim 21 wherein azathioprene is released from said composition by an enzyme-catalyzed release.

CW039P

23. The method of claim 21 wherein azathioprene is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

10 A composition comprising a polypeptide and azathioprene covalently attached to the polypeptide. Also provided is a method for delivery of azathioprene to a patient comprising administering to the patient a composition comprising a polypeptide and azathioprene covalently attached to the polypeptide. Also provided is a method for protecting azathioprene from degradation comprising covalently attaching it to a
15 polypeptide. Also provided is a method for controlling release of azathioprene from a composition comprising covalently attaching it to the polypeptide.

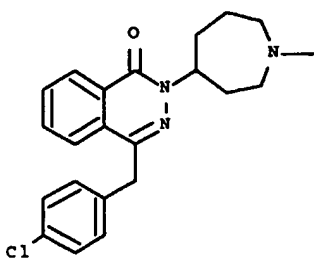
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING AZELASTINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to azelastine, as well as methods for protecting and administering azelastine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Azelastine is a known pharmaceutical agent that is used in the treatment of itching of the eye associated with allergic conjunctivitis. Its chemical name is 4-[(4-chlorophenyl)methyl]-2-(hexahydro-1-methyl-1H-azepin-4-yl)-1(2H)-phthalazinone. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

CW040P

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW040P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW040P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- 10 The present invention provides covalent attachment of the active agent (azelastine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching azelastine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
- 15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising azelastine microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and azelastine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
- 25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW040P

Azelastine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting azelastine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering azelastine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, azelastine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, azelastine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and azelastine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, azelastine is released

CW040P

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, azelastine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching azelastine to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, azelastine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

CW040P

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize azelastine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of azelastine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also
10 allows targeted delivery of active agents to specific sites of action.

Azelastine is the subject of U.S. Patent Number 5,164,194, herein incorporated by reference, which describes how to make that drug.

 The composition of the invention comprises azelastine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
15 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have
20 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

CW040P

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

CW040P

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

CW040P

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
 5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
 10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
 15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
 20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

CW040P

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW040P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-azelastine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW040P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW040P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW040P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 azelastine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein azelastine is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW040P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein azelastine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing azelastine from said composition in a pH-dependent manner.

15 19. A method for protecting azelastine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of azelastine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching azelastine to said polypeptide.

20 21. A method for delivering azelastine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
azelastine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein azelastine is released from said composition by an enzyme-catalyzed release.

CW040P

23. The method of claim 21 wherein azelastine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

A composition comprising a polypeptide and azelastine covalently attached to the polypeptide. Also provided is a method for delivery of azelastine to a patient comprising
15 administering to the patient a composition comprising a polypeptide and azelastine covalently attached to the polypeptide. Also provided is a method for protecting azelastine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of azelastine from a composition comprising covalently attaching it to the polypeptide.

CW041P

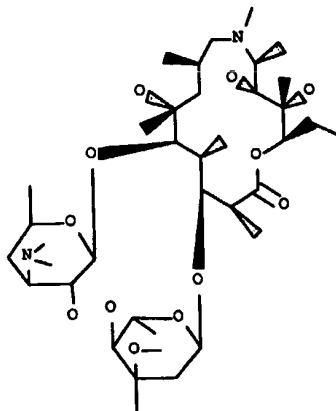
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING AZITHROMYCIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to azithromycin, as well as methods for protecting and administering azithromycin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Azithromycin is a known pharmaceutical agent that is used in the treatment of
15 bacterial infections. Its chemical name is (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-dideoxy-3-C-methyl-3-O-methyl- α -1-ribohexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,heptamethyl-11-[[[3,4,6-trideoxy-3-(dimethylamino) β -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability

CW041P

of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified

CW041P

amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that

CW041P

incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (azithromycin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching azithromycin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising azithromycin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and azithromycin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a

CW041P

heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

5 Azithromycin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is
10 an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The
15 microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be
20 conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

 The invention also provides a method for protecting azithromycin from degradation comprising covalently attaching it to a polypeptide.

25 The invention also provides a method for delivering azithromycin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, azithromycin is released from the composition

CW041P

by an enzyme-catalyzed release. In another preferred embodiment, azithromycin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and azithromycin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, azithromycin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, azithromycin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching azithromycin to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, azithromycin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a

CW041P

carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

5 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

10 The present invention provides several benefits for active agent delivery. First, the invention can stabilize azithromycin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of azithromycin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also
15 allows targeted delivery of active agents to specific sites of action.

Azithromycin is the subject of GB 2094293 B (1985), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises azithromycin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
20 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have
25 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The

CW041P

folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and

CW041P

at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

5 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

10 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

15 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

20 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

25 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain

CW041P

length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

CW041P

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to

CW041P

poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

5 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
10 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
15 carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
20 specific properties to the drug delivery system.

 The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
25 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
30 alimentary tract can affect release.

CW041P

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, azithromycin is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the

CW041P

mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-azithromycin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

CW041P

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
5 added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
10 solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

15 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

20 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product
25 precipitated out in ether and purified using GPC or dialysis.

CW041P

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW041P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 azithromycin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein azithromycin is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein azithromycin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing azithromycin from said composition in a pH-dependent manner.

15 19. A method for protecting azithromycin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of azithromycin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching azithromycin to said polypeptide.

20 21. A method for delivering azithromycin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
azithromycin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein azithromycin is released from said composition by an enzyme-catalyzed release.

CW041P

23. The method of claim 21 wherein azithromycin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

15 A composition comprising a polypeptide and azithromycin covalently attached to the polypeptide. Also provided is a method for delivery of azithromycin to a patient comprising administering to the patient a composition comprising a polypeptide and azithromycin covalently attached to the polypeptide. Also provided is a method for protecting azithromycin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of azithromycin from a composition comprising covalently attaching it to the polypeptide.

CW042P

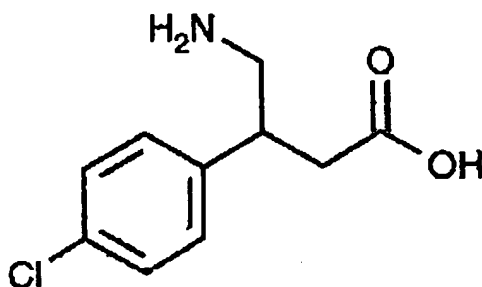
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING BACLOFEN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to baclofen, as well as methods for protecting and administering baclofen. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Baclofen is a known pharmaceutical agent that is used in the treatment of
15 spasticity. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW042P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW042P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW042P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (baclofen) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching baclofen to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising baclofen microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and baclofen covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Baclofen preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW042P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting baclofen from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering baclofen to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, baclofen is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, baclofen is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and baclofen is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, baclofen is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, baclofen is released from the composition in a sustained release.

CW042P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching baclofen to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, baclofen and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW042P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize baclofen and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of baclofen. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises baclofen covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW042P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

CW042P

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

CW042P

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

CW042P

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, baclofen is covalently attached to the polypeptide via the carboxylic acid group or the amine group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

CW042P

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-baclofen conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW042P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 baclofen covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein baclofen is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW042P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein baclofen is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing baclofen from said composition in a pH-dependent manner.

15 19. A method for protecting baclofen from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of baclofen from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching baclofen to said polypeptide.

20 21. A method for delivering baclofen to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
baclofen covalently attached to said polypeptide.

25 22. The method of claim 21 wherein baclofen is released from said composition by an enzyme-catalyzed release.

CW042P

23. The method of claim 21 wherein baclofen is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW042P

Abstract

A composition comprising a polypeptide and baclofen covalently attached to the polypeptide. Also provided is a method for delivery of baclofen to a patient comprising administering to the patient a composition comprising a polypeptide and baclofen covalently attached to the polypeptide. Also provided is a method for protecting baclofen from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of baclofen from a composition comprising covalently attaching it to the polypeptide:

5

CW043P

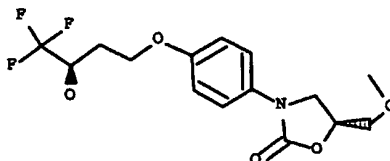
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING BEFLOXATONE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to befloxacitane, as well as methods for protecting and administering befloxacitane. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Befloxacitane is a known pharmaceutical agent that is used in smoking cessation treatment. Its chemical name is (R)-5-(methoxymethyl)-3-[4-[(R)-4,4,4-trifluoro-3-hydroxybutoxy]phenyl]-2-oxazolidinone. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW043P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW043P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some
5 technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically
10 require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the
15 gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The
20 released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that
25 incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly
30 or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW043P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (befloxatone) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching befloxatone to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising befloxatone microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and befloxatone covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Befloxatone preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW043P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting befloxacatone from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering befloxacatone to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, befloxacatone is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, befloxacatone is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and befloxacatone is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, befloxacatone is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, befloxacatone is released from the

CW043P

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching befloxacine to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, befloxacine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the
20 active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

CW043P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize befloxacitane and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of befloxacitane. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Befloxacitane is the subject of EP 424244 B (1995), herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises befloxacitane covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW043P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW043P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW043P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW043P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW043P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, befloxacone is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW043P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-befloxatone conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW043P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 befloxatone covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
15 one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein befloxatone is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
10. The composition of claim 9 wherein said microencapsulating agent is
20 selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW043P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein befloxtone is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing befloxtone from said composition in a pH-dependent manner.

15 19. A method for protecting befloxtone from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of befloxtone from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching befloxtone to said polypeptide.

20 21. A method for delivering befloxtone to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
befloxtone covalently attached to said polypeptide.

25 22. The method of claim 21 wherein befloxtone is released from said composition by an enzyme-catalyzed release.

CW043P

23. The method of claim 21 wherein befloxacatone is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and befloxacine covalently attached to the polypeptide. Also provided is a method for delivery of befloxacine to a patient comprising administering to the patient a composition comprising a polypeptide and befloxacine covalently attached to the polypeptide. Also provided is a method for protecting befloxacine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of befloxacine from a composition comprising covalently attaching it to the polypeptide.

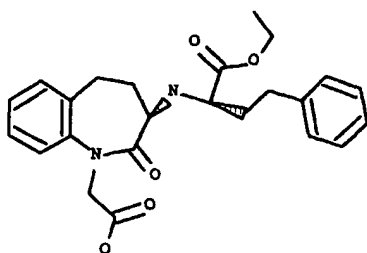
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING BENAZEPRIL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to benazepril, as well as methods for protecting and administering benazepril. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Benazepril has the chemical name [S-(R*,R*)]-3-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-acetic acid. It is
15 available commercially or can be made by those of skill in the art. Its structure is as follows:



20

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered

CW044P

product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

5 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another
10 invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

 Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of
15 cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme
20 degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

 Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example,
25 copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

CW044P

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

CW044P

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (benazepril) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching benazepril to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising benazepril microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and benazepril covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

CW044P

heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Benazepril preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a
5 carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-
10 terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
15 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
20 composition in a pH-dependent manner.

The invention also provides a method for protecting benazepril from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering benazepril to a patient, the patient being a human or a non-human animal, comprising administering to the patient a
25 composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, benazepril is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, benazepril is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed

CW044P

release. In another preferred embodiment, the composition further comprises a microencapsulating agent and benazepril is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, benazepril is released from the composition by a pH-dependent unfolding of the polypeptide. In another
5 preferred embodiment, benazepril is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

10 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching benazepril to a side chain of an amino acid to form an active agent/amino acid complex;
- 15 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
20 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, benazepril and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
25 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
30 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

CW044P

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize benazepril and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of benazepril. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Benazepril is the subject of U.S. Patent Number 4,410,520, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises benazepril covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW044P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW044P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW044P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW044P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW044P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, benazepril is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW044P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-benazepril conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW044P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

5 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in
15 ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with
20 triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of
25 other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW044P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW044P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW044P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 benazepril covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein benazepril is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW044P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein benazepril is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing benazepril from said composition in a pH-dependent manner.

15 19. A method for protecting benazepril from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of benazepril from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching benazepril to said polypeptide.

20 21. A method for delivering benazepril to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
benazepril covalently attached to said polypeptide.

25 22. The method of claim 21 wherein benazepril is released from said composition by an enzyme-catalyzed release.

CW044P

23. The method of claim 21 wherein benazepril is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

A composition comprising a polypeptide and benazepril covalently attached to the polypeptide. Also provided is a method for delivery of benazepril to a patient comprising
15 administering to the patient a composition comprising a polypeptide and benazepril covalently attached to the polypeptide. Also provided is a method for protecting benazepril from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of benazepril from a composition comprising covalently attaching it to the polypeptide.

CW045P

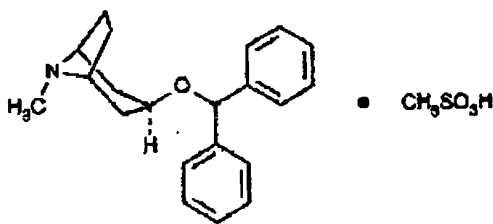
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING BENZATROPINE MESYLATE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to benztropine mesylate, as well as methods for protecting and administering benztropine mesylate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Benztropine mesylate is a known pharmaceutical agent that is used in the treatment of Parkinsoniajn syndrome. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

CW045P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW045P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW045P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (benzatropine mesylate) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching benzatropine mesylate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising benzatropine mesylate microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and benzatropine mesylate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Benzatropine mesylate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active

CW045P

agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet
5 another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino
10 acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In
15 another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting benzatropine mesylate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering benzatropine mesylate to a
20 patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, benzatropine mesylate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, benzatropine mesylate is released in a time-dependent manner based on the
25 pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and benzatropine mesylate is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, benzatropine mesylate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, benzatropine

CW045P

mesylate is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug
5 conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching benztropine mesylate to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
(NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, benztropine mesylate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

CW045P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize benztropine mesylate and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of benztropine mesylate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites
10 of action.

The composition of the invention comprises benztropine mesylate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino
15 acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and
20 turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on
25 the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the

CW045P

protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van
5 der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect
refers to the energetic consequences of removing apolar groups from the protein interior
and exposing them to water. Comparing the energy of amino acid hydrolysis with
protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular
bonds are formed at the expense of hydrogen bonds with water. Water molecules are
“pushed out” of the packed, hydrophobic protein core. All of these forces combine and
contribute to the overall stability of the folded protein where the degree to which ideal
packing occurs determines the degree of relative stability of the protein. The result of
15 maximum packing is to produce a center of residues or hydrophobic core that has
maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
peptide, it would require energy to unfold the peptide before the drug can be released.
The unfolding process requires overcoming the hydrophobic effect by hydrating the
20 amino acids or achieving the melting temperature of the protein. The heat of hydration is
a destabilization of a protein. Typically, the folded state of a protein is favored by only
5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
often observed prior to the onset of irreversible chemical or conformation processes.
25 Moreover, protein conformation generally controls the rate and extent of deleterious
chemical reactions.

Conformational protection of active agents by proteins depends on the stability of
the protein's folded state and the thermodynamics associated with the agent's

CW045P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW045P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW045P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW045P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, benztropine mesylate is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW045P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-benzatropine mesylate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW045P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW045P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW045P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 benzatropine mesylate covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein benzatropine mesylate is covalently
attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW045P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein benzatropine mesylate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing benzatropine mesylate from said composition in a pH-dependent manner.

15 19. A method for protecting benzatropine mesylate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of benzatropine mesylate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching benzatropine mesylate to said polypeptide.

20 21. A method for delivering benzatropine mesylate to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
benzatropine mesylate covalently attached to said polypeptide.

25 22. The method of claim 21 wherein benzatropine mesylate is released from said composition by an enzyme-catalyzed release.

CW045P

23. The method of claim 21 wherein benztropine mesylate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW046P

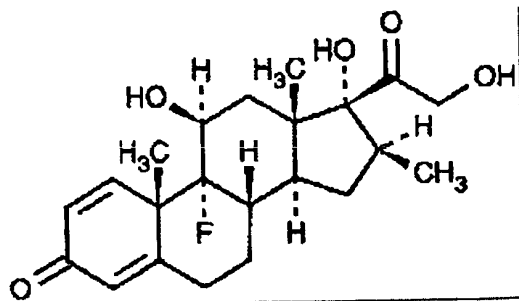
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING BETAMETHASONE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to betamethasone, as well as methods for protecting and administering betamethasone. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Betamethasone is a known pharmaceutical agent that is used principally as an anti-inflammatory or immunosuppressant agent. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW046P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW046P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent
10 (betamethasone) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching betamethasone to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach,
15 through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising betamethasone microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and betamethasone covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,
25 (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW046P

Betamethasone preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting betamethasone from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering betamethasone to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, betamethasone is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, betamethasone is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and betamethasone is released from the composition by dissolution of the microencapsulating agent. In another preferred

CW046P

embodiment, betamethasone is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, betamethasone is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching betamethasone to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, betamethasone and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

CW046P

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize betamethasone and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of betamethasone. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises betamethasone covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW046P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW046P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, betamethasone is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-betamethasone conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW046P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 betamethasone covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein betamethasone is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein betamethasone is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing betamethasone from said composition in a pH-dependent manner.

15 19. A method for protecting betamethasone from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of betamethasone from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching betamethasone to said polypeptide.

20 21. A method for delivering betamethasone to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
betamethasone covalently attached to said polypeptide.

25 22. The method of claim 21 wherein betamethasone is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein betamethasone is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and betamethasone covalently attached to the polypeptide. Also provided is a method for delivery of betamethasone to a patient comprising administering to the patient a composition comprising a polypeptide and
5 betamethasone covalently attached to the polypeptide. Also provided is a method for protecting betamethasone from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of betamethasone from a composition comprising covalently attaching it to the polypeptide.

CW047P

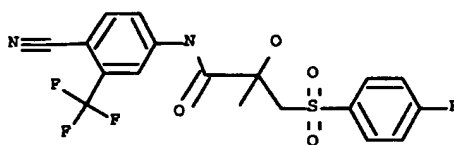
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING BICALUTAMIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to bicalutamide, as well as methods for protecting and administering bicalutamide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Bicalutamide is a known pharmaceutical agent that is used in the treatment of
15 locally advanced, non-metastatic prostate cancer, in combination with LHRH. Its chemical name is (+,-)-N-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methylpropanamide. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

CW047P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW047P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW047P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (bicalutamide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching bicalutamide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising bicalutamide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and bicalutamide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Bicalutamide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW047P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting bicalutamide from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering bicalutamide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, bicalutamide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, bicalutamide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and bicalutamide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, bicalutamide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, bicalutamide is released from the

CW047P

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching bicalutamide to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, bicalutamide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

CW047P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize bicalutamide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of bicalutamide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Bicalutamide is the subject of U.S. Patent Numbers 4,472,382, 4,636,505, and 5,389,613, herein incorporated by reference, which describes how to make that drug.

 The composition of the invention comprises bicalutamide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW047P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

• Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW047P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW047P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several

10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For

20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW047P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
30

CW047P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, bicalutamide is covalently attached to the polypeptide via the hydroxyl group. LHRH may also be attached to the same polypeptide to provide the two drugs in combination.

25 The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW047P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-bicalutamide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW047P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW047P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 bicalutamide covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein bicalutamide is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW047P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein bicalutamide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing bicalutamide from said composition in a pH-dependent manner.

15 19. A method for protecting bicalutamide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of bicalutamide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching bicalutamide to said polypeptide.

20 21. A method for delivering bicalutamide to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
bicalutamide covalently attached to said polypeptide.

25 22. The method of claim 21 wherein bicalutamide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein bicalutamide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and bicalutamide covalently attached to the polypeptide. Also provided is a method for delivery of bicalutamide to a patient comprising administering to the patient a composition comprising a polypeptide and

5 bicalutamide covalently attached to the polypeptide. Also provided is a method for protecting bicalutamide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of bicalutamide from a composition comprising covalently attaching it to the polypeptide.

CW048P

**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING BISOPROLOL
AND HYDROCHLOROTHIAZIDE AND METHODS OF
MAKING AND USING SAME**

5 FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to bisoprolol and hydrochlorothiazide, as well as methods for protecting and administering bisoprolol and hydrochlorothiazide. This novel compound, referred to as a CARRIERWAVE™
10 Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Bisoprolol and hydrochlorothiazide is a known pharmaceutical agent that is used in the treatment of -----. Its chemical name is -----. Its structure is: -----

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another

CW048P

invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

CW048P

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

CW048P

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (bisoprolol and hydrochlorothiazide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching
5 bisoprolol and hydrochlorothiazide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the
10 carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition
15 comprising bisoprolol and hydrochlorothiazide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and bisoprolol and hydrochlorothiazide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino
20 acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Bisoprolol and hydrochlorothiazide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment,
25 the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus

CW048P

of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The
5 microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be
10 conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting bisoprolol and hydrochlorothiazide from degradation comprising covalently attaching it to a
15 polypeptide.

The invention also provides a method for delivering bisoprolol and hydrochlorothiazide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment,
20 bisoprolol and hydrochlorothiazide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, bisoprolol and hydrochlorothiazide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and bisoprolol and hydrochlorothiazide is released from the
25 composition by dissolution of the microencapsulating agent. In another preferred embodiment, bisoprolol and hydrochlorothiazide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, bisoprolol and hydrochlorothiazide is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently

CW048P

attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching bisoprolol and hydrochlorothiazide to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, bisoprolol and hydrochlorothiazide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW048P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First,
5 the invention can stabilize bisoprolol and hydrochlorothiazide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of bisoprolol and hydrochlorothiazide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics
10 sites of action.

Bisoprolol and hydrochlorothiazide is the subject of U.S. Patent Number yyyyyy, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises bisoprolol and hydrochlorothiazide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
15 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The
25 folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding

CW048P

are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded
5 protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect
10 refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are
15 "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

20 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only
25 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

CW048P

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

25

CW048P

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

CW048P

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, bisoprolol and hydrochlorothiazide is covalently attached to the polypeptide via the zzzzzzz.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW048P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-bisoprolol and hydrochlorothiazide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW048P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW048P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW048P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 bisoprolol and hydrochlorothiazide covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein bisoprolol and hydrochlorothiazide is
covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW048P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein bisoprolol and hydrochlorothiazide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing bisoprolol and hydrochlorothiazide from said composition in a pH-dependent manner.

15 19. A method for protecting bisoprolol and hydrochlorothiazide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of bisoprolol and hydrochlorothiazide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching bisoprolol and hydrochlorothiazide to said polypeptide.

20 21. A method for delivering bisoprolol and hydrochlorothiazide to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
bisoprolol and hydrochlorothiazide covalently attached to said polypeptide.

25 22. The method of claim 21 wherein bisoprolol and hydrochlorothiazide is released from said composition by an enzyme-catalyzed release.

CW048P

23. The method of claim 21 wherein bisoprolol and hydrochlorothiazide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

f

CW048P

Abstract

A composition comprising a polypeptide and bisoprolol and hydrochlorothiazide covalently attached to the polypeptide. Also provided is a method for delivery of bisoprolol and hydrochlorothiazide to a patient comprising administering to the patient a
5 composition comprising a polypeptide and bisoprolol and hydrochlorothiazide covalently attached to the polypeptide. Also provided is a method for protecting bisoprolol and hydrochlorothiazide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of bisoprolol and hydrochlorothiazide from a composition comprising covalently attaching it to the
10 polypeptide.

CW049P

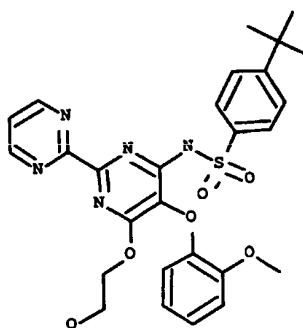
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING BOSENTAN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to bosentan, as well as methods for protecting and administering bosentan. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Bosentan is a known pharmaceutical agent that is used in the treatment of pulmonary hypertension. Its chemical name is 4-(1,1-dimethylethyl)-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)[2,2'-bipyrimidin]-4-yl]benzenesulfonamide. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

CW049P

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW049P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble
5 microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW049P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent (bosentan)
10 to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching bosentan to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection.
15 In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising bosentan microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and bosentan covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Bosentan preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting bosentan from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering bosentan to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, bosentan is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, bosentan is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and bosentan is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, bosentan is released

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, bosentan is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching bosentan to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, bosentan and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

CW049P

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize bosentan and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of bosentan. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted
10 delivery of active agents to specific sites of action.

Bosentan is the subject of EP 526708 A (1993), herein incorporated by reference, which describes how to make that drug.

 The composition of the invention comprises bosentan covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
15 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have
20 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior
10 and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

CW049P

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- Ionizing amino acids can be selected for pH controlled peptide unfolding.
- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
- 15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
- 20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
- 25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

CW049P

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

CW049P

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, bosentan is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW049P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-bosentan conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW049P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW049P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 bosentan covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein bosentan is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein bosentan is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing bosentan from said composition in a pH-dependent manner.

15 19. A method for protecting bosentan from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of bosentan from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching bosentan to said polypeptide.

20 21. A method for delivering bosentan to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
bosentan covalently attached to said polypeptide.

25 22. The method of claim 21 wherein bosentan is released from said composition by an enzyme-catalyzed release.

CW049P

23. The method of claim 21 wherein bosentan is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and bosentan covalently attached to the polypeptide. Also provided is a method for delivery of bosentan to a patient comprising administering to the patient a composition comprising a polypeptide and bosentan covalently attached to the polypeptide. Also provided is a method for protecting
5 bosentan from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of bosentan from a composition comprising covalently attaching it to the polypeptide.

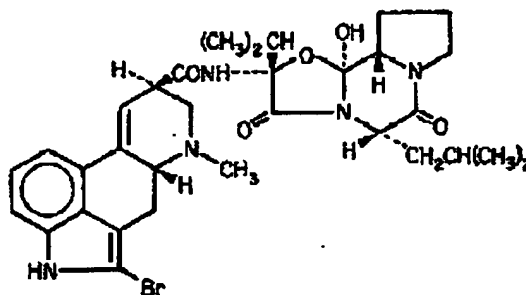
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING BROMOCRIPTINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to bromocriptine, as well as methods for protecting and administering bromocriptine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Bromocriptine is a known pharmaceutical agent that is used in the treatment of
15 dysfunctions associated with hyperprolactinemia including amenorrhea, with or without
galactorrhea; hypogonadism; and infertility. It is both commercially available and readily
manufactured using published synthetic schemes by those of ordinary skill in the art. Its
structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

CW050P

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW050P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW050P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent
10 (bromocriptine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching bromocriptine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach,
15 through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising bromocriptine microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and bromocriptine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,
25 (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW050P

Bromocriptine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting bromocriptine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering bromocriptine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, bromocriptine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, bromocriptine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and bromocriptine is released from the composition by dissolution of the microencapsulating agent. In another preferred

CW050P

embodiment, bromocriptine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, bromocriptine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching bromocriptine to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, bromocriptine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

CW050P

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize bromocriptine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of bromocriptine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises bromocriptine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW050P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW050P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, bromocriptine is covalently attached to the polypeptide via the hydroxyl group. Alternatively, it is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more
25 functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-bromocriptine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to
5 0°C. The solution can then be treated with diisopropylcarbodiimide and
hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be
stirred for several hours at room temperature, the urea by-product filtered off, the product
precipitated out in ether and purified using gel permeation chromatography (GPC) or
dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
followed by the amine bioactive agent. The reaction can then be stirred for several hours
at room temperature, the urea by-product filtered off, and the product precipitated out in
15 ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene
produces a chloroformate, which when reacted with the N-terminus of the peptide
produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with
20 triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
added slowly and the solution stirred at room temperature for several hours. The product
is then precipitated out in ether. The crude product is suitably deprotected and purified
using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of
25 other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
solvents such as chloroform. Examples of other activating agents include
dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW050P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 bromocriptine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein bromocriptine is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein bromocriptine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing bromocriptine from said composition in a pH-dependent manner.

15 19. A method for protecting bromocriptine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of bromocriptine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching bromocriptine to said polypeptide.

20 21. A method for delivering bromocriptine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
bromocriptine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein bromocriptine is released from said composition by an enzyme-catalyzed release.

CW050P

23. The method of claim 21 wherein bromocriptine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

 A composition comprising a polypeptide and bromocriptine covalently attached to the polypeptide. Also provided is a method for delivery of bromocriptine to a patient comprising administering to the patient a composition comprising a polypeptide and bromocriptine covalently attached to the polypeptide. Also provided is a method for
15 protecting bromocriptine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of bromocriptine from a composition comprising covalently attaching it to the polypeptide.

CW051P

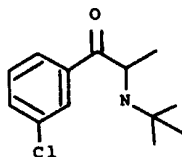
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING BUPROPION AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to bupropion, as well as methods for protecting and administering bupropion. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Bupropion is a known pharmaceutical agent that is used in smoking cessation
15 therapy and in the treatment of depression. Its chemical name is 1-(3-chlorophenyl)-2-[(1,1-dimethylethyl)amino]-1-propanone. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

CW051P

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

CW051P

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

CW051P

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (bupropion) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching bupropion to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

 Alternatively, the present invention provides a pharmaceutical composition comprising bupropion microencapsulated by a polypeptide.

 The invention provides a composition comprising a polypeptide and bupropion covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Bupropion preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

CW051P

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting bupropion from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering bupropion to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the
20 polypeptide. In a preferred embodiment, bupropion is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, bupropion is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and bupropion is released from the composition by dissolution
25 of the microencapsulating agent. In another preferred embodiment, bupropion is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, bupropion is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

CW051P

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

- (a) attaching bupropion to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and
- 10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, bupropion and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

CW051P

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize bupropion and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of bupropion.

5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Bupropion is the subject of U.S. Patent Numbers 5,358,970, 5,427,798, 5,731,000, 5,763,493, and Re. 33,994, herein incorporated by reference, which describes

10 how to make that drug.

The composition of the invention comprises bupropion covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a

15 heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and

20 turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on

25 the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the

CW051P

protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van
5 der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect
refers to the energetic consequences of removing apolar groups from the protein interior
and exposing them to water. Comparing the energy of amino acid hydrolysis with
protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular
bonds are formed at the expense of hydrogen bonds with water. Water molecules are
“pushed out” of the packed, hydrophobic protein core. All of these forces combine and
contribute to the overall stability of the folded protein where the degree to which ideal
packing occurs determines the degree of relative stability of the protein. The result of
15 maximum packing is to produce a center of residues or hydrophobic core that has
maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
peptide, it would require energy to unfold the peptide before the drug can be released.
The unfolding process requires overcoming the hydrophobic effect by hydrating the
20 amino acids or achieving the melting temperature of the protein. The heat of hydration is
a destabilization of a protein. Typically, the folded state of a protein is favored by only
5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
often observed prior to the onset of irreversible chemical or conformation processes.
25 Moreover, protein conformation generally controls the rate and extent of deleterious
chemical reactions.

Conformational protection of active agents by proteins depends on the stability of
the protein's folded state and the thermodynamics associated with the agent's

CW051P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW051P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW051P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW051P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, bupropion is covalently attached to the polypeptide via the amino group. Alternatively, it is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional
25 groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW051P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-bupropion conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW051P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW051P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW051P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 bupropion covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein bupropion is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW051P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.
- 5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein bupropion is conformationally protected by folding of said polypeptide about said active agent.
18. The composition of claim 1 wherein said polypeptide is capable of releasing bupropion from said composition in a pH-dependent manner.
- 15 19. A method for protecting bupropion from degradation comprising covalently attaching said active agent to a polypeptide.
20. A method for controlling release of bupropion from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching bupropion to said polypeptide.
- 20 21. A method for delivering bupropion to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
bupropion covalently attached to said polypeptide.
22. The method of claim 21 wherein bupropion is released from said composition
25 by an enzyme-catalyzed release.

CW051P

23. The method of claim 21 wherein bupropion is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

A composition comprising a polypeptide and bupropion covalently attached to the polypeptide. Also provided is a method for delivery of bupropion to a patient comprising
15 administering to the patient a composition comprising a polypeptide and bupropion covalently attached to the polypeptide. Also provided is a method for protecting bupropion from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of bupropion from a composition comprising covalently attaching it to the polypeptide.

CW052P

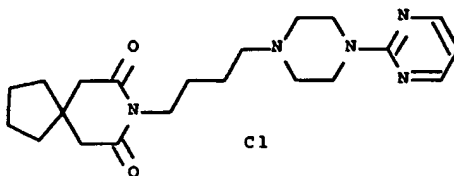
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING BUSPIRONE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to buspirone, as well as methods for protecting and administering buspirone. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Buspirone is a known pharmaceutical agent that is used in the treatment of atopic dermatitis. Its chemical name is 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4,5]decane-7,9-dione hydrochloride. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW052P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW052P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW052P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (buspirone) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching buspirone to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising buspirone microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and buspirone covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Buspirone preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW052P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting buspirone from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering buspirone to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, buspirone is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, buspirone is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and buspirone is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, buspirone is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, buspirone is released from the composition in a sustained release.

CW052P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching buspirone to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, buspirone and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW052P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize buspirone and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of buspirone. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Buspirone is the subject of U.S. Patent Number 4,182,763 and 5,015,646, herein incorporated by reference, which describes how to make that drug.

 The composition of the invention comprises buspirone covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW052P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW052P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW052P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW052P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
25 short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW052P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-buspirone conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW052P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 buspirone covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein buspirone is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein buspirone is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing buspirone from said composition in a pH-dependent manner.

15 19. A method for protecting buspirone from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of buspirone from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching buspirone to said polypeptide.

20 21. A method for delivering buspirone to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
buspirone covalently attached to said polypeptide.

25 22. The method of claim 21 wherein buspirone is released from said composition by an enzyme-catalyzed release.

CW052P

23. The method of claim 21 wherein buspirone is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW052P

Abstract

A composition comprising a polypeptide and buspirone covalently attached to the polypeptide. Also provided is a method for delivery of buspirone to a patient comprising administering to the patient a composition comprising a polypeptide and buspirone

5 covalently attached to the polypeptide. Also provided is a method for protecting buspirone from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of buspirone from a composition comprising covalently attaching it to the polypeptide.

CW053P

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING BUTORPHANOL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

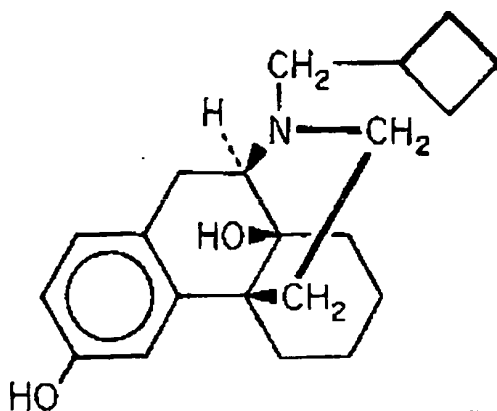
5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to butorphanol, as well as methods for protecting and administering butorphanol. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of

10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Butorphanol is a known pharmaceutical agent that is used in the treatment of pain.

15 It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability

20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;

CW053P

and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

CW053P

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

CW053P

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (butorphanol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching butorphanol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising butorphanol microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and butorphanol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

CW053P

heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Butorphanol preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In
5 another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-
10 terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
15 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
20 composition in a pH-dependent manner.

The invention also provides a method for protecting butorphanol from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering butorphanol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a
25 composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, butorphanol is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, butorphanol is released in a time-dependent manner based on the pharmacokinetics of the enzyme-

CW053P

catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and butorphanol is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, butorphanol is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, butorphanol is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching butorphanol to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, butorphanol and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the

CW053P

glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

- 5 The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- 10 The present invention provides several benefits for active agent delivery. First, the invention can stabilize butorphanol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of butorphanol. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

- 15 The composition of the invention comprises butorphanol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
20 more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
25 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW053P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW053P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW053P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW053P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW053P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, butorphanol is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW053P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-butorphanol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW053P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW053P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW053P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW053P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 butorphanol covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein butorphanol is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW053P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.
- 5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein butorphanol is conformationally protected by folding of said polypeptide about said active agent.
18. The composition of claim 1 wherein said polypeptide is capable of releasing butorphanol from said composition in a pH-dependent manner.
- 15 19. A method for protecting butorphanol from degradation comprising covalently attaching said active agent to a polypeptide.
20. A method for controlling release of butorphanol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching butorphanol to said polypeptide.
- 20 21. A method for delivering butorphanol to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
butorphanol covalently attached to said polypeptide.
22. The method of claim 21 wherein butorphanol is released from said
25 composition by an enzyme-catalyzed release.

CW053P

23. The method of claim 21 wherein butorphanol is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

15 A composition comprising a polypeptide and butorphanol covalently attached to the polypeptide. Also provided is a method for delivery of butorphanol to a patient comprising administering to the patient a composition comprising a polypeptide and butorphanol covalently attached to the polypeptide. Also provided is a method for protecting butorphanol from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of butorphanol from a composition comprising covalently attaching it to the polypeptide.

CW054P

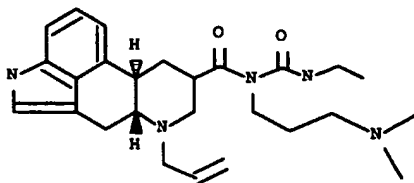
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CABERGOLINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cabergoline, as well as methods for protecting and administering cabergoline. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Cabergoline is a known pharmaceutical agent that is used in the treatment of Parkinson's disease. Its chemical name is (8beta)-N-[3-(dimethylamino)propyl]-N-[(ethylamino)carbonyl]-6-(2-prop enyl)ergoline-8-carboxamide. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW054P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW054P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW054P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cabergoline) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cabergoline to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cabergoline microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cabergoline covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cabergoline preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW054P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cabergoline from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cabergoline to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cabergoline is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cabergoline is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cabergoline is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cabergoline is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cabergoline is released from the

CW054P

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cabergoline to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cabergoline and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the
20 active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

CW054P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cabergoline and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cabergoline. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Cabergoline is the subject of U.S. Patent Number 4,526,892, herein incorporated by reference, which describes how to make that drug.

 The composition of the invention comprises cabergoline covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW054P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW054P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW054P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW054P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW054P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cabergoline is covalently attached to the polypeptide via the amino group. Alternatively, it can be covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more
25 functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW054P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cabergoline conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW054P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW054P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

5 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW054P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW054P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cabergoline covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cabergoline is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW054P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cabergoline is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cabergoline from said composition in a pH-dependent manner.

15 19. A method for protecting cabergoline from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cabergoline from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cabergoline to said polypeptide.

20 21. A method for delivering cabergoline to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cabergoline covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cabergoline is released from said composition by an enzyme-catalyzed release.

CW054P

23. The method of claim 21 wherein cabergoline is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

A composition comprising a polypeptide and cabergoline covalently attached to the polypeptide. Also provided is a method for delivery of cabergoline to a patient
15 comprising administering to the patient a composition comprising a polypeptide and cabergoline covalently attached to the polypeptide. Also provided is a method for protecting cabergoline from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cabergoline from a composition comprising covalently attaching it to the polypeptide.

CW055P

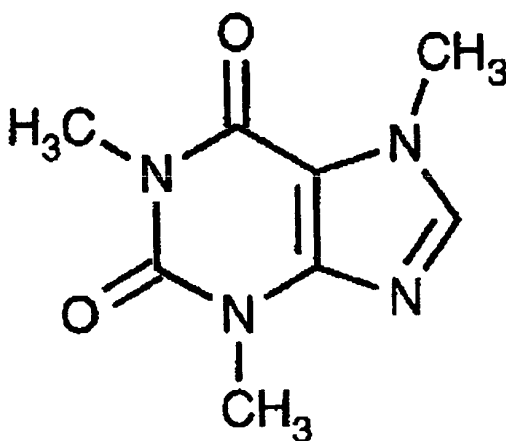
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CAFFEINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to caffeine, as well as methods for protecting and administering caffeine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Caffeine is a known pharmaceutical agent that is used in the treatment of neonatal
15 apnea. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;

CW055P

and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

CW055P

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

CW055P

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (caffeine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching caffeine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising caffeine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and caffeine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

CW055P

heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Caffeine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a
5 carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-
10 terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
15 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
20 composition in a pH-dependent manner.

The invention also provides a method for protecting caffeine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering caffeine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a
25 composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, caffeine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, caffeine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release.

CW055P

In another preferred embodiment, the composition further comprises a microencapsulating agent and caffeine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, caffeine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, caffeine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

10 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching caffeine to a side chain of an amino acid to form an active agent/amino acid complex;
- 15 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, caffeine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

CW055P

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize caffeine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of caffeine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises caffeine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW055P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
5 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine,
10 lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is
15 important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the
25 kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW055P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW055P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW055P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
25 short chain of either amino acids or carbohydrates

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-caffeine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW055P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW055P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 caffeine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein caffeine is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein caffeine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing caffeine from said composition in a pH-dependent manner.

15 19. A method for protecting caffeine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of caffeine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching caffeine to said polypeptide.

20 21. A method for delivering caffeine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
caffeine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein caffeine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein caffeine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and caffeine covalently attached to the polypeptide. Also provided is a method for delivery of caffeine to a patient comprising administering to the patient a composition comprising a polypeptide and caffeine covalently attached to the polypeptide. Also provided is a method for protecting caffeine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of caffeine from a composition comprising covalently attaching it to the polypeptide.

CW056P

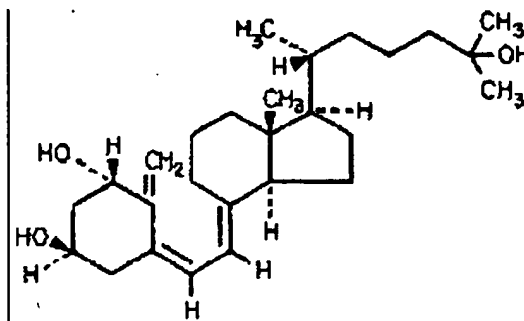
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CALCITRIOL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to calcitriol, as well as methods for protecting and administering calcitriol. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Calcitriol is a known pharmaceutical agent that is used in the treatment of
15 hypocalcemia. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW056P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW056P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW056P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (calcitriol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching calcitriol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising calcitriol microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and calcitriol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Calcitriol preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW056P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting calcitriol from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering calcitriol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, calcitriol is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, calcitriol is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and calcitriol is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, calcitriol is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, calcitriol is released from the composition in a sustained release.

CW056P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching calcitriol to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, calcitriol and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW056P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize calcitriol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of calcitriol. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Calcitriol is the subject of U.S. Patent Numbers 4,308,264 and 6,051,567, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises calcitriol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW056P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW056P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW056P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW056P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW056P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, calcitriol is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW056P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-calcitriol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW056P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW056P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW056P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 calcitriol covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein calcitriol is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW056P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.
- 5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
16. The composition of claim 1 wherein said composition is in the form of an
10 oral suspension.
17. The composition of claim 1 wherein calcitriol is conformationally protected by folding of said polypeptide about said active agent.
18. The composition of claim 1 wherein said polypeptide is capable of releasing calcitriol from said composition in a pH-dependent manner.
- 15 19. A method for protecting calcitriol from degradation comprising covalently attaching said active agent to a polypeptide.
20. A method for controlling release of calcitriol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching calcitriol to said polypeptide.
- 20 21. A method for delivering calcitriol to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
calcitriol covalently attached to said polypeptide.
22. The method of claim 21 wherein calcitriol is released from said composition
25 by an enzyme-catalyzed release.

CW056P

23. The method of claim 21 wherein calcitriol is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW056P

Abstract

A composition comprising a polypeptide and calcitriol covalently attached to the polypeptide. Also provided is a method for delivery of calcitriol to a patient comprising administering to the patient a composition comprising a polypeptide and calcitriol covalently attached to the polypeptide. Also provided is a method for protecting calcitriol from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of calcitriol from a composition comprising covalently attaching it to the polypeptide.

CW057P

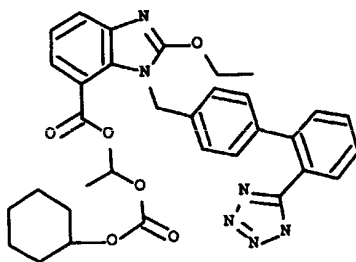
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CANDESARTAN CILEXITIL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to candesartan cilexetil, as well as methods for protecting and administering candesartan cilexetil. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Candesartan cilexetil is a known pharmaceutical agent that is used in the treatment of heart failure. Its chemical name is 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1-H-benzimidazole-7-carboxylic acid 1-[[[(cyclohexyloxy)carbonyl]oxy]ethyl ester. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

CW057P

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW057P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW057P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent
10 (candesartan cilexetil) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching candesartan cilexetil to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the
15 stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising candesartan cilexetil microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and candesartan cilexetil covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,
25 (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW057P

Candesartan cilexetil preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting candesartan cilexetil from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering candesartan cilexetil to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, candesartan cilexetil is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, candesartan cilexetil is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and candesartan cilexetil is released from the composition by dissolution of the microencapsulating agent. In another

CW057P

preferred embodiment, candesartan cilexetil is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, candesartan cilexetil is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching candesartan cilexetil to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, candesartan cilexetil and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

CW057P

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize candesartan cilexetil and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of candesartan cilexetil. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Candesartan cilexetil is the subject of U.S. Patent Numbers 5,196,444, 5,534,534, 5,703,110 and 5,705,517, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises candesartan cilexetil covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW057P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW057P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW057P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW057P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

CW057P

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

5 The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex,
10 PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
15 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
20 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
25 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

CW057P

Preferably, the resultant peptide-candesartan cilexetil conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

5 **Acid/N-terminus conjugation**

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product
10 precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
15 followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene
20 produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified
25 using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated

CW057P

solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

5 Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered,
10 dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for
15 several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes
20 homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically
25 overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

CW057P

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW057P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 candesartan cilexetil covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein candesartan cilexetil is covalently attached
to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW057P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein candesartan cilexetil is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing candesartan cilexetil from said composition in a pH-dependent manner.

15 19. A method for protecting candesartan cilexetil from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of candesartan cilexetil from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching candesartan cilexetil to said polypeptide.

20 21. A method for delivering candesartan cilexetil to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
candesartan cilexetil covalently attached to said polypeptide.

25 22. The method of claim 21 wherein candesartan cilexetil is released from said composition by an enzyme-catalyzed release.

CW057P

23. The method of claim 21 wherein candesartan cilexetil is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

A composition comprising a polypeptide and candesartan cilexetil covalently attached to the polypeptide. Also provided is a method for delivery of candesartan cilexetil to a patient comprising administering to the patient a composition comprising a
15 polypeptide and candesartan cilexetil covalently attached to the polypeptide. Also provided is a method for protecting candesartan cilexetil from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of candesartan cilexetil from a composition comprising covalently attaching it to the polypeptide.

CW058P

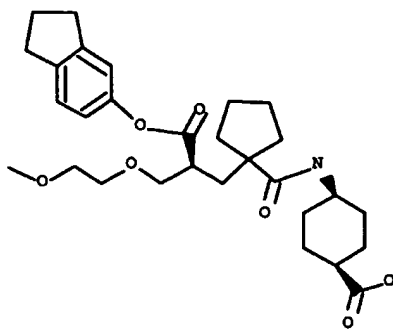
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CANDOXATRIL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to candoxatril, as well as methods for protecting and administering candoxatril. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Candoxatril is a known pharmaceutical agent that is used in the treatment of heart
15 failure and hypertension. Its chemical name is 4-[[[1-[3-[(2,3-dihydro-1H-inden-5-yl)oxy]-2-[(2-methoxyethoxy)methyl]-3-oxopropyl]cyclopentyl]carbonyl]amino]-cyclohexanecarboxylic acid. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

CW058P

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW058P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW058P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent
10 (candoxatril) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching candoxatril to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising candoxatril microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and candoxatril covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW058P

Candoxatril preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting candoxatril from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering candoxatril to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, candoxatril is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, candoxatril is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and candoxatril is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, candoxatril is

CW058P

released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, candoxatril is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching candoxatril to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, candoxatril and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

CW058P

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000,
5 incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize candoxatril and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of candoxatril.
10 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Candoxatril is the subject of EP 274234 B (1991), herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises candoxatril covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
20 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
25 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW058P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW058P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW058P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW058P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, candoxatril is covalently attached to the polypeptide via the carboxylic acid.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW058P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-candoxatril conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW058P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW058P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW058P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 candoxatril covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein candoxatril is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW058P

12.. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein candoxatril is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing candoxatril from said composition in a pH-dependent manner.

15 19. A method for protecting candoxatril from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of candoxatril from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching candoxatril to said polypeptide.

20 21. A method for delivering candoxatril to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
candoxatril covalently attached to said polypeptide.

25 22. The method of claim 21 wherein candoxatril is released from said composition by an enzyme-catalyzed release.

CW058P

23. The method of claim 21 wherein candoxatril is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

A composition comprising a polypeptide and candoxatril covalently attached to the polypeptide. Also provided is a method for delivery of candoxatril to a patient
15 comprising administering to the patient a composition comprising a polypeptide and candoxatril covalently attached to the polypeptide. Also provided is a method for protecting candoxatril from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of candoxatril from a composition comprising covalently attaching it to the polypeptide.

CW059P

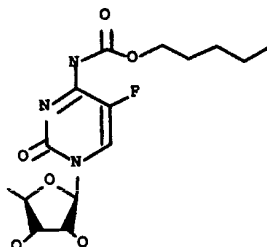
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CAPECITABINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to capecitabine, as well as methods for protecting and administering capecitabine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Capecitabine is a known pharmaceutical agent that is used in the treatment of
15 colorectal cancer. Its chemical name is pentyl 1-(5-deoxy-.beta.-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinecarbamate. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW059P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example,

20 copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW059P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW059P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (capecitabine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching capecitabine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising capecitabine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and capecitabine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Capecitabine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW059P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting capecitabine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering capecitabine to a patient; the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, capecitabine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, capecitabine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and capecitabine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, capecitabine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, capecitabine is released from the

CW059P

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching capecitabine to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, capecitabine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

CW059P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize capecitabine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of capecitabine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Capecitabine is the subject of U.S. Patent Numbers 4,966,891 and 5,472,949, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises capecitabine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW059P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW059P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW059P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW059P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW059P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, capecitabine is covalently attached to the polypeptide via the hydroxyl groups.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW059P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-capecitabine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW059P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW059P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW059P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 capecitabine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
15 one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein capecitabine is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
10. The composition of claim 9 wherein said microencapsulating agent is
20 selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW059P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein capecitabine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing capecitabine from said composition in a pH-dependent manner.

15 19. A method for protecting capecitabine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of capecitabine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching capecitabine to said polypeptide.

20 21. A method for delivering capecitabine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
capecitabine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein capecitabine is released from said composition by an enzyme-catalyzed release.

CW059P

23. The method of claim 21 wherein capecitabine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW059P

Abstract

A composition comprising a polypeptide and capecitabine covalently attached to the polypeptide. Also provided is a method for delivery of capecitabine to a patient comprising administering to the patient a composition comprising a polypeptide and capecitabine covalently attached to the polypeptide. Also provided is a method for
5 protecting capecitabine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of capecitabine from a composition comprising covalently attaching it to the polypeptide.

CW060P

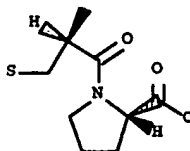
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CAPTOPRIL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to captopril, as well as methods for protecting and administering captopril. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Captopril is a known pharmaceutical agent that is used in the treatment of
15 hypertension. Its chemical name is 1-(3-mercapto-2-methyl-1-oxopropyl)-L-proline. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

CW060P

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

CW060P

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

CW060P

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (captopril) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching captopril to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will
10 stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a
15 second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising captopril microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and captopril covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Captopril preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

CW060P

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting captopril from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering captopril to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, captopril is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, captopril is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and captopril is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, captopril is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, captopril is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

20

25

CW060P

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching captopril to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, captopril and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

CW060P

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize captopril and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of captopril. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Captopril is the subject of U.S. Patent Number 5,238,924, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises captopril covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW060P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

CW060P

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

CW060P

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW060P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

CW060P

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, captopril is covalently attached to the polypeptide via the carboxylic acid.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

CW060P

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
5 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the
10 transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active
15 agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the
20 catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-captopril conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

25 Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW060P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW060P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW060P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 captopril covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein captopril is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW060P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein captopril is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing captopril from said composition in a pH-dependent manner.

15 19. A method for protecting captopril from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of captopril from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching captopril to said polypeptide.

20 21. A method for delivering captopril to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
captopril covalently attached to said polypeptide.

25 22. The method of claim 21 wherein captopril is released from said composition by an enzyme-catalyzed release.

CW060P

23. The method of claim 21 wherein captopril is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW060P

Abstract

A composition comprising a polypeptide and captopril covalently attached to the polypeptide. Also provided is a method for delivery of captopril to a patient comprising administering to the patient a composition comprising a polypeptide and captopril

5 covalently attached to the polypeptide. Also provided is a method for protecting captopril from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of captopril from a composition comprising covalently attaching it to the polypeptide.

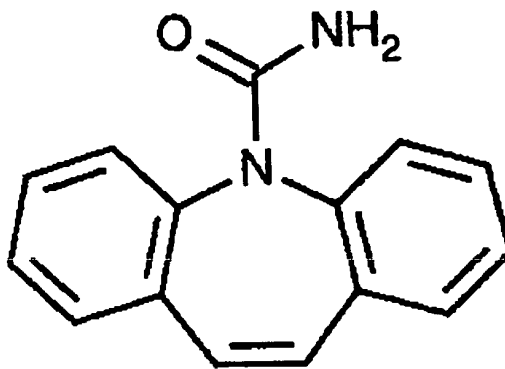
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CARBAMAZEPINE

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to carbamazepine, as well as methods for protecting and administering carbamazepine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Carbamazepine is a known pharmaceutical agent that is used in the treatment of
15 epilepsy. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

CW061P

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.

20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

30

CW061P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
10 (carbamazepine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching carbamazepine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach,
15 through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising carbamazepine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and carbamazepine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,
25 (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Carbamazepine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting carbamazepine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering carbamazepine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, carbamazepine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, carbamazepine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and carbamazepine is released from the composition by dissolution of the microencapsulating agent. In another preferred

CW061P

embodiment, carbamazepine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, carbamazepine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching carbamazepine to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, carbamazepine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000,
5 incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize carbamazepine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of
10 carbamazepine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Carbamazepine is the subject of U.S. Patent Numbers 5,284,662 and Re. 34,990, herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises carbamazepine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
20 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
25 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW061P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW061P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, carbamazepine is covalently attached to the polypeptide via the amido group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW061P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-carbamazepine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW061P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to
5 0°C. The solution can then be treated with diisopropylcarbodiimide and
hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be
stirred for several hours at room temperature, the urea by-product filtered off, the product
precipitated out in ether and purified using gel permeation chromatography (GPC) or
dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
followed by the amine bioactive agent. The reaction can then be stirred for several hours
at room temperature, the urea by-product filtered off, and the product precipitated out in
15 ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene
produces a chloroformate, which when reacted with the N-terminus of the peptide
produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with
20 triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
added slowly and the solution stirred at room temperature for several hours. The product
is then precipitated out in ether. The crude product is suitably deprotected and purified
using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of
25 other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
solvents such as chloroform. Examples of other activating agents include
dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW061P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 carbamazepine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein carbamazepine is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein carbamazepine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing carbamazepine from said composition in a pH-dependent manner.

15 19. A method for protecting carbamazepine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of carbamazepine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching carbamazepine to said polypeptide.

20 21. A method for delivering carbamazepine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
carbamazepine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein carbamazepine is released from said composition by an enzyme-catalyzed release.

CW061P

23. The method of claim 21 wherein carbamazepine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

A composition comprising a polypeptide and carbamazepine covalently attached to the polypeptide. Also provided is a method for delivery of carbamazepine to a patient
15 comprising administering to the patient a composition comprising a polypeptide and carbamazepine covalently attached to the polypeptide. Also provided is a method for protecting carbamazepine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of carbamazepine from a composition comprising covalently attaching it to the polypeptide.

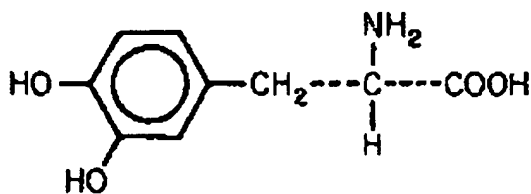
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CARBIDOPA AND LEVODOPA

FIELD OF THE INVENTION

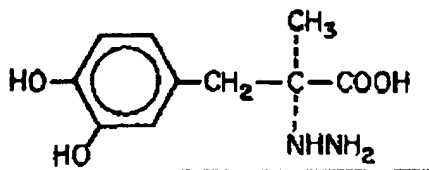
5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to carbidopa and levodopa, as well as methods for protecting and administering carbidopa and levodopa. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Carbidopa and levodopa are known pharmaceutical agents that are used together in the treatment of Parkinson's disease. Each is commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Carbidopa's structure is:



Levodopa's structure is:



20

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability

CW062P

of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified

CW062P

amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several
5 shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent
10 in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as
15 pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous
20 pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where
25 the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide
30 linker. Thus, there has been no drug delivery system, heretofore reported, that

CW062P

incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (carbidopa and levodopa) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching carbidopa and levodopa to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising carbidopa and levodopa microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and carbidopa and levodopa covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,

CW062P

(iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

5 Carbidopa and levodopa preferably are covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent
10 is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The
15 microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be
20 conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

 The invention also provides a method for protecting carbidopa and levodopa from degradation comprising covalently attaching it to a polypeptide.

25 The invention also provides a method for delivering carbidopa and levodopa to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, carbidopa and levodopa is

released from the composition by an enzyme-catalyzed release. In another preferred embodiment, carbidopa and levodopa is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and carbidopa and
5 levodopa is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, carbidopa and levodopa is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, carbidopa and levodopa is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an
10 adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
15 comprises the steps of:

- (a) attaching carbidopa and levodopa to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- 20 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, carbidopa and levodopa and a second active agent can be copolymerized in step
25 (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is
30 attached to the side chain of the amino acid to form an amide, a thioester, an ester, an

CW062P

ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

5 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

10 DETAILED DESCRIPTION OF INVENTION

 The present invention provides several benefits for active agent delivery. First, the invention can stabilize carbidopa and levodopa and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of carbidopa and levodopa. Furthermore, active agents can be combined to produce
15 synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

 The composition of the invention comprises carbidopa and levodopa covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a
20 homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have
25 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The

folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and

CW062P

at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

5 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

 Selection of the amino acids will depend on the physical properties desired. For
10 instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
15 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
20 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
25 synergistic effect between two or more active agents is desired.

 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain

CW062P

length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to

CW062P

poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

- 5 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety
10 from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
15 any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
20 specific properties to the drug delivery system.

- The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
25 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
30 alimentary tract can affect release.

CW062P

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are
5 known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, carbidopa and levodopa are each covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A
10 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG)
15 and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
20 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the
25 transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the

mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-carbidopa and levodopa conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

CW062P

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
5 added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
10 solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

15 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

20 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product
25 precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product,
5 which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and
10 filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 carbidopa and levodopa covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein carbidopa and levodopa is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein carbidopa and levodopa is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing carbidopa and levodopa from said composition in a pH-dependent manner.

15 19. A method for protecting carbidopa and levodopa from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of carbidopa and levodopa from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching carbidopa and levodopa to said polypeptide.

20 21. A method for delivering carbidopa and levodopa to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
carbidopa and levodopa covalently attached to said polypeptide.

25 22. The method of claim 21 wherein carbidopa and levodopa is released from said composition by an enzyme-catalyzed release.

CW062P

23. The method of claim 21 wherein carbidopa and levodopa is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 **Abstract**

 A composition comprising a polypeptide and carbidopa and levodopa covalently attached to the polypeptide. Also provided is a method for delivery of carbidopa and levodopa to a patient comprising administering to the patient a composition comprising a polypeptide and carbidopa and levodopa covalently attached to the polypeptide. Also
15 provided is a method for protecting carbidopa and levodopa from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of carbidopa and levodopa from a composition comprising covalently attaching it to the polypeptide.

CW063P

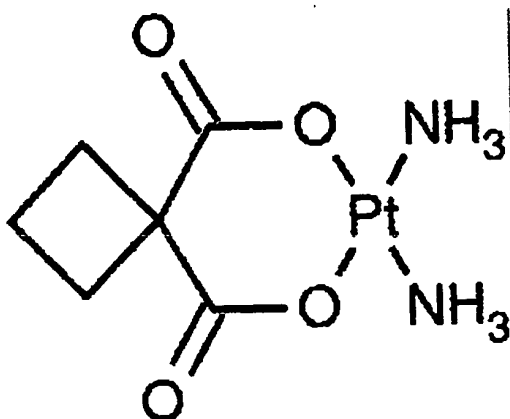
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CARBOPLATIN

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises
5 a polypeptide that is preferably covalently attached to carboplatin, as well as methods for
protecting and administering carboplatin. This novel compound, referred to as a
CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known
effective pharmaceutical agent that is both well studied and occupies a known segment of
the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical
effectiveness.

BACKGROUND OF THE INVENTION

Carboplatin is a known pharmaceutical agent that is used in the treatment of
ovarian cancer. It is both commercially available and readily manufactured using
15 published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical

CW063P

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW063P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW063P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent
10 (carboplatin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching carboplatin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising carboplatin microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and carboplatin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW063P

Carboplatin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting carboplatin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering carboplatin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, carboplatin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, carboplatin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and carboplatin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, carboplatin is

CW063P

released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, carboplatin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching carboplatin to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, carboplatin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

CW063P

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize carboplatin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of carboplatin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises carboplatin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW063P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW063P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW063P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, carboplatin is covalently attached to the polypeptide via the amine groups.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW063P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-carboplatin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW063P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 carboplatin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein carboplatin is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.
- 5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein carboplatin is conformationally protected by folding of said polypeptide about said active agent.
18. The composition of claim 1 wherein said polypeptide is capable of releasing carboplatin from said composition in a pH-dependent manner.
- 15 19. A method for protecting carboplatin from degradation comprising covalently attaching said active agent to a polypeptide.
20. A method for controlling release of carboplatin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching carboplatin to said polypeptide.
- 20 21. A method for delivering carboplatin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
carboplatin covalently attached to said polypeptide.
22. The method of claim 21 wherein carboplatin is released from said
25 composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein carboplatin is released from said composition by a pH-dependent unfolding of said polypeptide.
24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.
- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and carboplatin covalently attached to the polypeptide. Also provided is a method for delivery of carboplatin to a patient comprising administering to the patient a composition comprising a polypeptide and carboplatin covalently attached to the polypeptide. Also provided is a method for protecting carboplatin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of carboplatin from a composition comprising covalently attaching it to the polypeptide.

CW064P

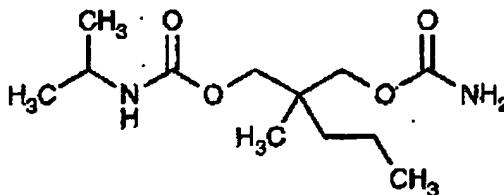
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CARISOPRODOL

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to carisoprodol, as well as methods for protecting and administering carisoprodol. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Carisoprodol is a known pharmaceutical agent that is used in the treatment of
15 skeletal muscle spasm. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW064P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some
5 technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically
10 require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the
15 gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The
20 released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that
25 incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly
30 or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW064P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (carisoprodol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching carisoprodol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising carisoprodol microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and carisoprodol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Carisoprodol preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting carisoprodol from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering carisoprodol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, carisoprodol is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, carisoprodol is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and carisoprodol is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, carisoprodol is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, carisoprodol is released from the

CW064P

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching carisoprodol to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, carisoprodol and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

CW064P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize carisoprodol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of carisoprodol. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises carisoprodol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW064P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.
- 25

CW064P

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW064P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, carisoprodol is covalently attached to the polypeptide via the amido group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
5 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the
10 transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active
15 agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the
20 catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-carisoprodol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

25 Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 carisoprodol covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein carisoprodol is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW064P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein carisoprodol is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing carisoprodol from said composition in a pH-dependent manner.

15 19. A method for protecting carisoprodol from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of carisoprodol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching carisoprodol to said polypeptide.

20 21. A method for delivering carisoprodol to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
carisoprodol covalently attached to said polypeptide.

25 22. The method of claim 21 wherein carisoprodol is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein carisoprodol is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and carisoprodol covalently attached to the polypeptide. Also provided is a method for delivery of carisoprodol to a patient comprising administering to the patient a composition comprising a polypeptide and carisoprodol covalently attached to the polypeptide. Also provided is a method for protecting carisoprodol from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of carisoprodol from a composition comprising covalently attaching it to the polypeptide.

CW065P

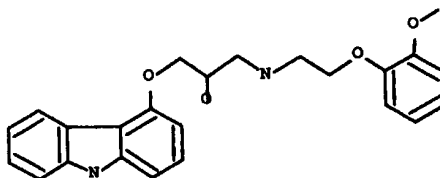
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CARVEDILOL

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises
5 a polypeptide that is preferably covalently attached to carvedilol, as well as methods for
protecting and administering carvedilol. This novel compound, referred to as a
CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known
effective pharmaceutical agent that is both well studied and occupies a known segment of
the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical
effectiveness.

BACKGROUND OF THE INVENTION

Carvedilol is a known pharmaceutical agent that is used in the treatment of heart
failure. Its chemical name is 1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)
15 ethyl]amino]-2-propanol. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical
compound may contain one or more of the following: another active pharmaceutical
agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a
25 biologically active agent (active agent) to the appropriate target. The importance of these

CW065P

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system: Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

CW065P

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (carvedilol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching carvedilol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

 Alternatively, the present invention provides a pharmaceutical composition comprising carvedilol microencapsulated by a polypeptide.

 The invention provides a composition comprising a polypeptide and carvedilol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Carvedilol preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting carvedilol from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering carvedilol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, carvedilol is released from the composition by
20 an enzyme-catalyzed release. In another preferred embodiment, carvedilol is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and carvedilol is released from the composition by dissolution
25 of the microencapsulating agent. In another preferred embodiment, carvedilol is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, carvedilol is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

- (a) attaching carvedilol to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- 10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, carvedilol and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize carvedilol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of carvedilol.

5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Carvedilol is the subject of U.S. Patent Numbers 4,503,067, 5,760,069, and 5,902,821, herein incorporated by reference, which describes how to make that drug.

10 The composition of the invention comprises carvedilol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or

15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the

20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding

25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW065P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW065P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

CW065P

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, carvedilol is covalently attached to the polypeptide via the hydrozyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-carvedilol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments,
the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 carvedilol covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein carvedilol is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.
- 5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein carvedilol is conformationally protected by folding of said polypeptide about said active agent.
18. The composition of claim 1 wherein said polypeptide is capable of releasing carvedilol from said composition in a pH-dependent manner.
- 15 19. A method for protecting carvedilol from degradation comprising covalently attaching said active agent to a polypeptide.
20. A method for controlling release of carvedilol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching carvedilol to said polypeptide.
- 20 21. A method for delivering carvedilol to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
carvedilol covalently attached to said polypeptide.
22. The method of claim 21 wherein carvedilol is released from said composition
25 by an enzyme-catalyzed release.

23. The method of claim 21 wherein carvedilol is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and carvedilol covalently attached to the polypeptide. Also provided is a method for delivery of carvedilol to a patient comprising administering to the patient a composition comprising a polypeptide and carvedilol covalently attached to the polypeptide. Also provided is a method for protecting carvedilol from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of carvedilol from a composition comprising covalently attaching it to the polypeptide.

5

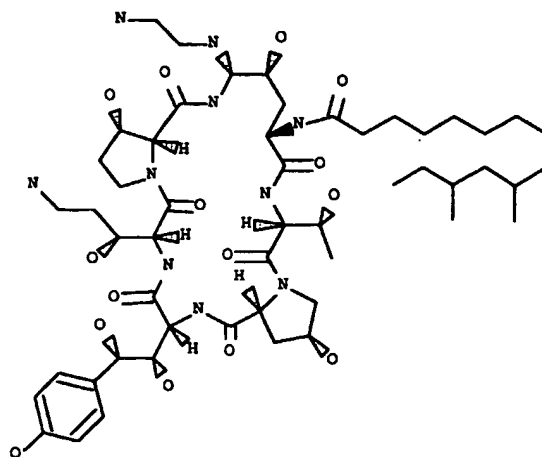
CW066P

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CASPOFUNGIN**FIELD OF THE INVENTION**

The present invention relates to a novel pharmaceutical compound that comprises
5 a polypeptide that is preferably covalently attached to caspofungin, as well as methods
for protecting and administering caspofungin. This novel compound, referred to as a
CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known
effective pharmaceutical agent that is both well studied and occupies a known segment of
the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical
effectiveness.

BACKGROUND OF THE INVENTION

Caspofungin is a known pharmaceutical agent that is used in the treatment of
bacterial and fungal infections. Its chemical name is 1-[(4R,5S)-5-[(2-
15 aminoethyl)amino]-N2-(10,12-dimethyl-1-oxotetradecyl)-4-hydroxy-L-ornithine]-5-
[(3R)-3-hydroxy-L-ornithine]pneumocandin B0. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered

product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

- 5 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another
- 10 invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

- Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of
- 15 cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme
- 20 degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

- Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example,
- 25 copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

CW066P

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

CW066P

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (caspofungin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching caspofungin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising caspofungin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and caspofungin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Caspofungin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a
5 carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-
10 terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
15 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
20 composition in a pH-dependent manner.

The invention also provides a method for protecting caspofungin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering caspofungin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a
25 composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, caspofungin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, caspofungin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-

CW066P

catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and caspofungin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, caspofungin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, caspofungin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching caspofungin to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, caspofungin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the

glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

- 5 The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- 10 The present invention provides several benefits for active agent delivery. First, the invention can stabilize caspofungin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of caspofungin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

- 15 Caspofungin is the subject of WO 94/21677 (1994), based on U.S. Patent Application Number 32847 (1993), herein incorporated by reference, which describes how to make that drug.

- 20 The composition of the invention comprises caspofungin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

- 25 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The

folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and

CW066P

at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

5 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

 Selection of the amino acids will depend on the physical properties desired. For
10 instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
15 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
20 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
25 synergistic effect between two or more active agents is desired.

 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain

length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to

poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

5 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
10 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
15 carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
20 specific properties to the drug delivery system.

 The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
25 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
30 alimentary tract can affect release.

CW066P

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
5 dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, caspofungin is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A
10 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG)
15 and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
20 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the
25 transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the

CW066P

mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-caspofungin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

CW066P

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
5 added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
10 solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

15 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

20 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product
25 precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 caspofungin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein caspofungin is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein caspofungin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing caspofungin from said composition in a pH-dependent manner.

15 19. A method for protecting caspofungin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of caspofungin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching caspofungin to said polypeptide.

20 21. A method for delivering caspofungin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
caspofungin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein caspofungin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein caspofungin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

 A composition comprising a polypeptide and caspofungin covalently attached to the polypeptide. Also provided is a method for delivery of caspofungin to a patient comprising administering to the patient a composition comprising a polypeptide and caspofungin covalently attached to the polypeptide. Also provided is a method for
15 protecting caspofungin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of caspofungin from a composition comprising covalently attaching it to the polypeptide.

CW067P

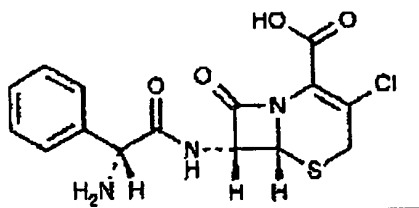
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFACLOR

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises
5 a polypeptide that is preferably covalently attached to cefaclor, as well as methods for
protecting and administering cefaclor. This novel compound, referred to as a
CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known
effective pharmaceutical agent that is both well studied and occupies a known segment of
the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical
effectiveness.

BACKGROUND OF THE INVENTION

Cefaclor is a known pharmaceutical agent that is used in the treatment of
bronchitis. It is both commercially available and readily manufactured using published
15 synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical
compound may contain one or more of the following: another active pharmaceutical
agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a
25 biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (cefaclor) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefaclor to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will
10 stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a
15 second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cefaclor microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cefaclor covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Cefaclor preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting cefaclor from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering cefaclor to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cefaclor is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cefaclor is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cefaclor is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cefaclor is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cefaclor is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

20

25

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

- (a) attaching cefaclor to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and
- 10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefaclor and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize cefaclor and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cefaclor. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises cefaclor covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

CW067P

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

CW067P

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

20

CW067P

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

CW067P

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cefaclor is covalently attached to the polypeptide via the carboxylic acid.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

CW067P

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-cefaclor conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

CW067P

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

CW067P

several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- 20 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cefaclor covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cefaclor is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cefaclor is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cefaclor from said composition in a pH-dependent manner.

15 19. A method for protecting cefaclor from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cefaclor from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cefaclor to said polypeptide.

20 21. A method for delivering cefaclor to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cefaclor covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cefaclor is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cefaclor is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cefaclor covalently attached to the polypeptide. Also provided is a method for delivery of cefaclor to a patient comprising administering to the patient a composition comprising a polypeptide and cefaclor covalently attached to the polypeptide. Also provided is a method for protecting cefaclor from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cefaclor from a composition comprising covalently attaching it to the polypeptide.

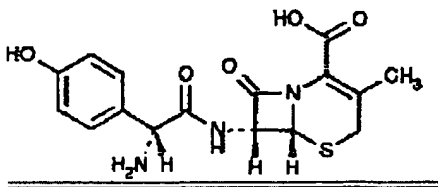
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFADROXIL

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cefadroxil, as well as methods for protecting and administering cefadroxil. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Cefadroxil is a known pharmaceutical agent that is used in the treatment of bacterial infections. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a
25 biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (cefadroxil) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefadroxil to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cefadroxil microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cefadroxil covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Cefadroxil preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting cefadroxil from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering cefadroxil to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the
20 polypeptide. In a preferred embodiment, cefadroxil is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cefadroxil is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cefadroxil is released from the composition by dissolution
25 of the microencapsulating agent. In another preferred embodiment, cefadroxil is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cefadroxil is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

- (a) attaching cefadroxil to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefadroxil and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize cefadroxil and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cefadroxil.

- 5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

- The composition of the invention comprises cefadroxil covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
10 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have
15 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
20 constitute the tertiary structure.

- Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino
25 acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
 5 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
 15 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
 20 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cefadroxil is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

CW068P

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-cefadroxil conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- 20 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cefadroxil covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cefadroxil is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cefadroxil is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cefadroxil from said composition in a pH-dependent manner.

15 19. A method for protecting cefadroxil from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cefadroxil from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cefadroxil to said polypeptide.

20 21. A method for delivering cefadroxil to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cefadroxil covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cefadroxil is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cefadroxil is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cefadroxil covalently attached to the polypeptide. Also provided is a method for delivery of cefadroxil to a patient comprising administering to the patient a composition comprising a polypeptide and cefadroxil

5 covalently attached to the polypeptide. Also provided is a method for protecting cefadroxil from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cefadroxil from a composition comprising covalently attaching it to the polypeptide.

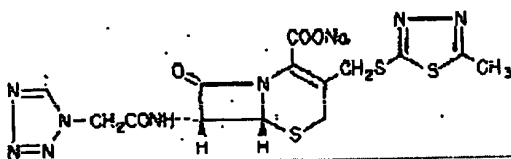
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFAZOLIN

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises
5 a polypeptide that is preferably covalently attached to cefazolin, as well as methods for
protecting and administering cefazolin. This novel compound, referred to as a
CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known
effective pharmaceutical agent that is both well studied and occupies a known segment of
the pharmaceutical market; and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical
effectiveness.

BACKGROUND OF THE INVENTION

Cefazolin is a known pharmaceutical agent that is used in the treatment of
respiratory tract infections, urinary tract infections, skin and skin structure infections,
15 biliary tract infections, bone and joint infections, genital infections, septicemia, and
endocarditis caused by susceptible bacteria. It is both commercially available and readily
manufactured using published synthetic schemes by those of ordinary skill in the art. Its
structure is:



20 The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
of the original compound; alteration of the release profile of an orally administered
product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical
25 compound may contain one or more of the following: another active pharmaceutical
agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW069P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cefazolin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefazolin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cefazolin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cefazolin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cefazolin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cefazolin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cefazolin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cefazolin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cefazolin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cefazolin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cefazolin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cefazolin is released from the composition in a sustained release.

CW069P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

 (a) attaching cefazolin to a side chain of an amino acid to form an active agent/amino acid complex;

10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefazolin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cefazolin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cefazolin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises cefazolin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW069P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, cefazolin is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

CW069P

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cefazolin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cefazolin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cefazolin is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW069P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cefazolin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cefazolin from said composition in a pH-dependent manner.

15 19. A method for protecting cefazolin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cefazolin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cefazolin to said polypeptide.

20 21. A method for delivering cefazolin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cefazolin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cefazolin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cefazolin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cefazolin covalently attached to the polypeptide. Also provided is a method for delivery of cefazolin to a patient comprising administering to the patient a composition comprising a polypeptide and cefazolin covalently attached to the polypeptide. Also provided is a method for protecting cefazolin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cefazolin from a composition comprising covalently attaching it to the polypeptide.

CW070P

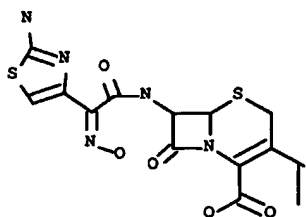
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFDINIR

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises
5 a polypeptide that is preferably covalently attached to cefdinir, as well as methods for
protecting and administering cefdinir. This novel compound, referred to as a
CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known
effective pharmaceutical agent that is both well studied and occupies a known segment of
the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical
effectiveness.

BACKGROUND OF THE INVENTION

Cefdinir is a known pharmaceutical agent that is used in the treatment of acute
maxillary sinusitis, acute exacerbations of chronic bronchitis, pharyngitis, tonsillitis,
15 community-acquired pneumonia and bacterial skin infections. Its chemical name is [6R-
[6alpha,7beta(Z)]]-7-[[[(2-amino-4-thiazolyl)(hydroxyimino) acetyl]amino]-3-ethenyl-8-
oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability
of the original compound; alteration of the release profile of an orally administered
product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical
compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW070P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW070P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cefdinir) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefdinir to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cefdinir microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cefdinir covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cefdinir preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic

CW070P

acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred
5 embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino
10 acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In
15 another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cefdinir from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cefdinir to a patient, the
20 patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cefdinir is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cefdinir is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release.
25 In another preferred embodiment, the composition further comprises a microencapsulating agent and cefdinir is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cefdinir is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cefdinir is released from the composition in a sustained release. In yet

CW070P

another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cefdinir to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefdinir and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefdinir and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW070P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cefdinir and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cefdinir. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Cefdinir is the subject of EP 105459 B (1989), based on US Application Serial Number 428,970 (1982) and EP 304019 B (1995), herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises cefdinir covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

CW070P

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

CW070P

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

CW070P

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cefdinir is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW070P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cefdinir conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW070P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW070P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cefdinir covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cefdinir is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cefdinir is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cefdinir from said composition in a pH-dependent manner.

15 19. A method for protecting cefdinir from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cefdinir from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cefdinir to said polypeptide.

20 21. A method for delivering cefdinir to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cefdinir covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cefdinir is released from said composition by an enzyme-catalyzed release.

CW070P

23. The method of claim 21 wherein cefdinir is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW070P

Abstract

A composition comprising a polypeptide and cefdinir covalently attached to the polypeptide. Also provided is a method for delivery of cefdinir to a patient comprising administering to the patient a composition comprising a polypeptide and cefdinir covalently attached to the polypeptide. Also provided is a method for protecting cefdinir from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cefdinir from a composition comprising covalently attaching it to the polypeptide.

CW071P

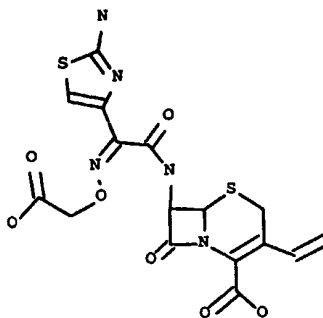
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFIXIME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises
5 a polypeptide that is preferably covalently attached to cefixime, as well as methods for
protecting and administering cefixime. This novel compound, referred to as a
CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known
effective pharmaceutical agent that is both well studied and occupies a known segment of
the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical
effectiveness.

BACKGROUND OF THE INVENTION

Cefixime is a known pharmaceutical agent that is used in the treatment of
respiratory tract infections, gonorrhea, biliary tract infection and pediatric otitis media.
15 Its chemical name is [6R-[6alpha,7beta(Z)]]-7-[[[(2-amino-4-thiazolyl)](carboxymethoxy)
imino]acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic
acid. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability
of the original compound; alteration of the release profile of an orally administered
product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical

CW071P

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW071P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble
5 microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.

20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW071P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent (cefixime) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefixime to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection.
- 10 to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefixime to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection.
- 15 In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising cefixime microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and cefixime covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
- 25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW071P

Cefixime preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cefixime from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cefixime to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cefixime is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cefixime is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cefixime is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cefixime is released

CW071P

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cefixime is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cefixime to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefixime and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

CW071P

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cefixime and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cefixime. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted
10 delivery of active agents to specific sites of action.

Cefixime is the subject of EP 30360 B (1987), herein incorporated by reference, which describes how to make that drug.

 The composition of the invention comprises cefixime covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
15 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have
20 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

CW071P

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior
10 and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

CW071P

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- Ionizing amino acids can be selected for pH controlled peptide unfolding.
- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
- 15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
- 20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
- 25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

CW071P

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

CW071P

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

CW071P

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine,
10 polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be
15 attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the
20 attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cefixime is covalently attached to the polypeptide via the carboxylic acid group.

25 The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW071P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-cefixime conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW071P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW071P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW071P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cefixime covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cefixime is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW071P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cefixime is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cefixime from said composition in a pH-dependent manner.

15 19. A method for protecting cefixime from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cefixime from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cefixime to said polypeptide.

20 21. A method for delivering cefixime to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cefixime covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cefixime is released from said composition by an enzyme-catalyzed release.

CW071P

23. The method of claim 21 wherein cefixime is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW071P

Abstract

A composition comprising a polypeptide and cefixime covalently attached to the polypeptide. Also provided is a method for delivery of cefixime to a patient comprising administering to the patient a composition comprising a polypeptide and cefixime covalently attached to the polypeptide. Also provided is a method for protecting cefixime from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cefixime from a composition comprising covalently attaching it to the polypeptide.

CW072P

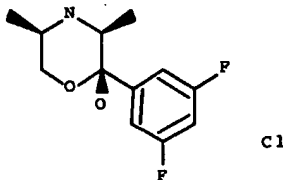
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING NORADRENALIN AND DOPAMINE REUPTAKE INHIBITOR

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to the noradrenalin and dopamine reuptake inhibitor, as well as methods for protecting and administering the noradrenalin and dopamine reuptake inhibitor. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known
10 effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 The noradrenalin and dopamine reuptake inhibitor of the present invention is used in the treatment of attention deficit hyperactivity disorder (ADHA). Its chemical name is [2S-(2alpha,3alpha,5alpha)]-2-(3,5-difluorophenyl)-3,5-dimethyl-2-morpholinol hydrochloride. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical
25 compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW072P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW072P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW072P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (noradrenalin and dopamine reuptake inhibitor) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching the noradrenalin and dopamine reuptake inhibitor to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising the noradrenalin and dopamine reuptake inhibitor microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and the noradrenalin and dopamine reuptake inhibitor covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW072P

The noradrenalin and dopamine reuptake inhibitor preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting the noradrenalin and dopamine reuptake inhibitor from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering the noradrenalin and dopamine reuptake inhibitor to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, the noradrenalin and dopamine reuptake inhibitor is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, the noradrenalin and dopamine reuptake inhibitor is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the

CW072P

composition further comprises a microencapsulating agent and the noradrenalin and dopamine reuptake inhibitor is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, the noradrenalin and dopamine reuptake inhibitor is released from the composition by a pH-dependent
5 unfolding of the polypeptide. In another preferred embodiment, the noradrenalin and dopamine reuptake inhibitor is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier
10 peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching the noradrenalin and dopamine reuptake inhibitor to a side chain of an amino
15 acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

20 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, the noradrenalin and dopamine reuptake inhibitor and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis
25 of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an
30 ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant

CW072P

group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

- 5 The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- 10 The present invention provides several benefits for active agent delivery. First, the invention can stabilize the noradrenalin and dopamine reuptake inhibitor and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of the noradrenalin and dopamine reuptake inhibitor. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery
15 of active agents to specific sites of action.

The noradrenalin and dopamine reuptake inhibitor of the present invention is the subject of EP 426416 B (1994), herein incorporated by reference, which describes how to make that drug.

- 20 The composition of the invention comprises the noradrenalin and dopamine reuptake inhibitor covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more
25 synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and

CW072P

turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

5 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the
10 protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
15 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
20 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
25 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is

CW072P

a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

- 5 Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different
10 than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 15 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
20 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 25 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

CW072P

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

CW072P

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to

CW072P

poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

5 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
10 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
15 carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
20 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
25 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
30 alimentary tract can affect release.

CW072P

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the noradrenalin and dopamine reuptake inhibitor is covalently attached to the polypeptide via the hydroxyl.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the

CW072P

mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-noradrenalin and dopamine reuptake inhibitor conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

CW072P

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

15 Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

CW072P

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

15

CW072P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 noradrenalin and dopamine reuptake inhibitor covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
- 10 4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
15 two or more synthetic amino acids.
7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein the noradrenalin and dopamine reuptake inhibitor is covalently attached to a side chain, the N-terminus or the C-terminus of said
20 polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

CW072P

11. The composition of claim 1 further comprising an adjuvant.
12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable
5 excipient.
14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein the noradrenalin and dopamine reuptake inhibitor is conformationally protected by folding of said polypeptide about said active agent.
- 15 18. The composition of claim 1 wherein said polypeptide is capable of releasing the noradrenalin and dopamine reuptake inhibitor from said composition in a pH-dependent manner.
19. A method for protecting the noradrenalin and dopamine reuptake inhibitor from degradation comprising covalently attaching said active agent to a polypeptide.
- 20 20. A method for controlling release of the noradrenalin and dopamine reuptake inhibitor from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching the noradrenalin and dopamine reuptake inhibitor to said polypeptide.
21. A method for delivering the noradrenalin and dopamine reuptake inhibitor to
25 a patient comprising administering to said patient a composition comprising:

CW072P

a polypeptide; and

the noradrenalin and dopamine reuptake inhibitor covalently attached to said polypeptide.

22. The method of claim 21 wherein the noradrenalin and dopamine reuptake inhibitor is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein the noradrenalin and dopamine reuptake inhibitor is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

15 A composition comprising a polypeptide and the noradrenalin and dopamine reuptake inhibitor covalently attached to the polypeptide. Also provided is a method for delivery of the noradrenalin and dopamine reuptake inhibitor to a patient comprising administering to the patient a composition comprising a polypeptide and the noradrenalin and dopamine reuptake inhibitor covalently attached to the polypeptide. Also provided is
20 a method for protecting the noradrenalin and dopamine reuptake inhibitor from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of the noradrenalin and dopamine reuptake inhibitor from a composition comprising covalently attaching it to the polypeptide.

CW073P

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFOTAXIME**FIELD OF THE INVENTION**

The present invention relates to a novel pharmaceutical compound that comprises
5 a polypeptide that is preferably covalently attached to cefotaxime, as well as methods for
protecting and administering cefotaxime. This novel compound, referred to as a
CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known
effective pharmaceutical agent that is both well studied and occupies a known segment of
the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical
effectiveness.

BACKGROUND OF THE INVENTION

Cefotaxime is a known pharmaceutical agent that is used in the treatment of
serious bone and joint infections, serious intra-abdominal and gynecologic infections
15 (including peritonitis, endometritis, pelvic inflammatory disease, pelvic cellulitis),
meningitis and other CNS infections, serious lower respiratory tract infections (including
pneumonia), bacteremia/septicemia, serious skin and skin structure infections, and
serious urinary tract infections caused by susceptible bacteria. It is both commercially
available and readily manufactured using published synthetic schemes by those of
20 ordinary skill in the art. Its chemical name is (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)
glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,
7²(*Z*)-[O-(carboxymethyl)oxime].

The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
25 of the original compound; alteration of the release profile of an orally administered
product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical
compound may contain one or more of the following: another active pharmaceutical
agent, an adjuvant, or an inhibitor.

CW073P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW073P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW073P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cefotaxime) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefotaxime to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cefotaxime microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cefotaxime covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cefotaxime preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW073P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cefotaxime from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cefotaxime to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cefotaxime is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cefotaxime is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cefotaxime is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cefotaxime is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cefotaxime is released from the composition in a

CW073P

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cefotaxime to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefotaxime and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW073P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cefotaxime and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cefotaxime. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises cefotaxime covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW073P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

CW073P

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

CW073P

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW073P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

CW073P

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, cefotaxime is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

CW073P

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cefotaxime conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW073P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW073P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW073P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cefotaxime covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cefotaxime is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW073P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cefotaxime is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cefotaxime from said composition in a pH-dependent manner.

15 19. A method for protecting cefotaxime from degradation comprising covalently attaching said active agent to a polypeptide.

20 20. A method for controlling release of cefotaxime from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cefotaxime to said polypeptide.

20 21. A method for delivering cefotaxime to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cefotaxime covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cefotaxime is released from said composition by an enzyme-catalyzed release.

CW073P

23. The method of claim 21 wherein cefotaxime is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW073P

Abstract

A composition comprising a polypeptide and cefotaxime covalently attached to the polypeptide. Also provided is a method for delivery of cefotaxime to a patient comprising administering to the patient a composition comprising a polypeptide and cefotaxime covalently attached to the polypeptide. Also provided is a method for protecting cefotaxime from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cefotaxime from a composition comprising covalently attaching it to the polypeptide.

CW074P

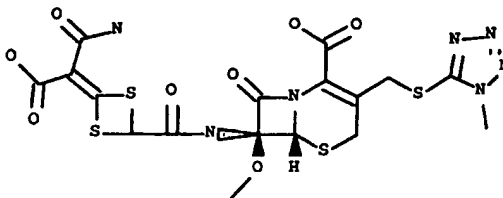
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFOTETAN

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises
 5 a polypeptide that is preferably covalently attached to cefotetan, as well as methods for
 protecting and administering cefotetan. This novel compound, referred to as a
 CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known
 effective pharmaceutical agent that is both well studied and occupies a known segment of
 the pharmaceutical market, and combining it with a carrier compound that enhances the
 10 usefulness of the pharmaceutical agent without compromising its pharmaceutical
 effectiveness.

BACKGROUND OF THE INVENTION

Cefotetan is a known pharmaceutical agent that is used in the treatment of
 septicemia, genitourinary, biliary and respiratory tract infections, and in
 15 postoperativewound infection prophylaxis. Its chemical name is [6R-(6 α ,7 α)]-7-
 [[[4-(2-amino-1-carboxy-2-oxoethylidene)-1,3-dithietan-2-yl]carbonyl]amino]-7-
 methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio] methyl]-8-oxo-5-thia-1-azabicyclo
 [4.2.0]oct-2-ene-2-carboxylic acid. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in
 accomplishing one or more of the following goals: enhancement of the chemical stability
 of the original compound; alteration of the release profile of an orally administered
 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
 and provision for an oral dosage form when none exists. The novel pharmaceutical

CW074P

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW074P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble
5 microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW074P

- diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.
- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent (cefotetan) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefotetan to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection.
- 15 In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising cefotetan microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and cefotetan covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
- 25

CW074P

Cefotetan preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cefotetan from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cefotetan to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cefotetan is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cefotetan is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cefotetan is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cefotetan is released

CW074P

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cefotetan is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is
5 controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- 10 (a) attaching cefotetan to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
15 (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefotetan and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released
20 from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side
25 chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the
30 following detailed description are exemplary, but are not restrictive, of the invention.

CW074P

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cefotetan and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cefotetan. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted
10 delivery of active agents to specific sites of action.

Cefotetan is the subject of GB 1604739 (1981), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises cefotetan covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
15 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have
20 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

CW074P

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

CW074P

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

CW074P

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
 5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
 10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
 15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
 20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

CW074P

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

CW074P

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cefotetan is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW074P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-cefotetan conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW074P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW074P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW074P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cefotetan covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cefotetan is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW074P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cefotetan is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cefotetan from said composition in a pH-dependent manner.

15 19. A method for protecting cefotetan from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cefotetan from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cefotetan to said polypeptide.

20 21. A method for delivering cefotetan to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cefotetan covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cefotetan is released from said composition by an enzyme-catalyzed release.

CW074P

23. The method of claim 21 wherein cefotetan is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW074P

Abstract

A composition comprising a polypeptide and cefotetan covalently attached to the polypeptide. Also provided is a method for delivery of cefotetan to a patient comprising administering to the patient a composition comprising a polypeptide and cefotetan covalently attached to the polypeptide. Also provided is a method for protecting cefotetan from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cefotetan from a composition comprising covalently attaching it to the polypeptide.

CW075P

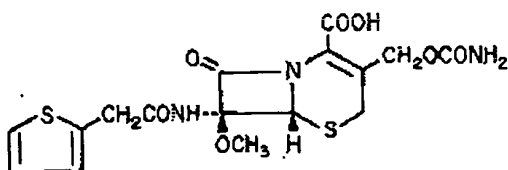
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFOTAXIME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises
5 a polypeptide that is preferably covalently attached to cefotaxime, as well as methods for
protecting and administering cefotaxime. This novel compound, referred to as a
CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known
effective pharmaceutical agent that is both well studied and occupies a known segment of
the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical
effectiveness.

BACKGROUND OF THE INVENTION

Cefotaxime is a known pharmaceutical agent that is used in the treatment of serious
infections of the lower respiratory tract, skin and skin structure, bone and joint, and
15 urinary tract; septicemia; gynecologic infections (including endometritis, pelvic cellulitis,
and pelvic inflammatory disease); and intra-abdominal infections (including peritonitis
and intra-abdominal abscess) caused by susceptible bacteria. It is both commercially
available and readily manufactured using published synthetic schemes by those of
ordinary skill in the art. Its structure is:



20

The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
of the original compound; alteration of the release profile of an orally administered
product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
25 and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent (cefoxitin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefoxitin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection.
- 15 In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising cefoxitin microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and cefoxitin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
- 25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cefoxitin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cefoxitin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cefoxitin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cefoxitin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cefoxitin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cefoxitin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cefoxitin is released

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cefoxitin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is
5 controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- 10 (a) attaching cefoxitin to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
15 (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefoxitin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released
20 from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side
25 chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the
30 following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cefoxitin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cefoxitin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted
10 delivery of active agents to specific sites of action.

 The composition of the invention comprises cefoxitin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a
15 heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and
20 turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on
25 the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the

protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van
5 der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect
refers to the energetic consequences of removing apolar groups from the protein interior
and exposing them to water. Comparing the energy of amino acid hydrolysis with
protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular
bonds are formed at the expense of hydrogen bonds with water. Water molecules are
“pushed out” of the packed, hydrophobic protein core. All of these forces combine and
contribute to the overall stability of the folded protein where the degree to which ideal
packing occurs determines the degree of relative stability of the protein. The result of
15 maximum packing is to produce a center of residues or hydrophobic core that has
maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
peptide, it would require energy to unfold the peptide before the drug can be released.
The unfolding process requires overcoming the hydrophobic effect by hydrating the
20 amino acids or achieving the melting temperature of the protein. The heat of hydration is
a destabilization of a protein. Typically, the folded state of a protein is favored by only
5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
often observed prior to the onset of irreversible chemical or conformation processes.
25 Moreover, protein conformation generally controls the rate and extent of deleterious
chemical reactions.

Conformational protection of active agents by proteins depends on the stability of
the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
30

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cefoxitin is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-cefoxitin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW075P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cefoxitin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cefoxitin is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cefoxitin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cefoxitin from said composition in a pH-dependent manner.

15 19. A method for protecting cefoxitin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cefoxitin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cefoxitin to said polypeptide.

20 21. A method for delivering cefoxitin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cefoxitin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cefoxitin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cefoxitin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cefoxitin covalently attached to the polypeptide. Also provided is a method for delivery of cefoxitin to a patient comprising administering to the patient a composition comprising a polypeptide and cefoxitin covalently attached to the polypeptide. Also provided is a method for protecting
5 cefoxitin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cefoxitin from a composition comprising covalently attaching it to the polypeptide.

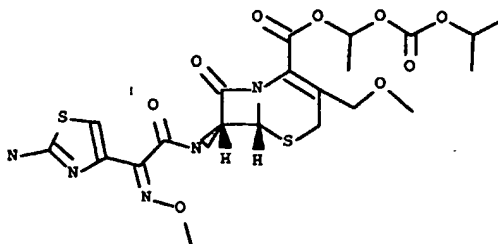
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFPODOXIME PROXETIL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cefpodoxime proxetil, as well as methods for protecting and administering cefpodoxime proxetil. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Cefpodoxime proxetil is a known pharmaceutical agent that is used in the
15 treatment of mild to moderate infections of the upper and lower respiratory tract, skin and urinary tract and sexually transmitted diseases. Its chemical name is [6R-[6 α ,7 β (Z)]]-7-[[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 1-[[[(1-methylethoxy)carbonyl]oxy]ethyl ester. Its structure is:



20

 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;

and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

CW076P

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

CW076P

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cefpodoxime proxetil) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefpodoxime proxetil to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cefpodoxime proxetil microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cefpodoxime proxetil covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or

(vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cefpodoxime proxetil preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active
5 agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to
10 the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
15 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
20 composition in a pH-dependent manner.

The invention also provides a method for protecting cefpodoxime proxetil from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cefpodoxime proxetil to a patient, the patient being a human or a non-human animal, comprising administering to
25 the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cefpodoxime proxetil is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cefpodoxime proxetil is released in a time-dependent manner based on the

pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cefpodoxime proxetil is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cefpodoxime proxetil is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cefpodoxime proxetil is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cefpodoxime proxetil to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefpodoxime proxetil and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the

glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

- 5 The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- The present invention provides several benefits for active agent delivery. First, 10 the invention can stabilize cefpodoxime proxetil and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cefpodoxime proxetil. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites 15 of action.

Cefpodoxime proxetil is the subject of EP 49118 B (1986), herein incorporated by reference, which describes how to make that drug.

- The composition of the invention comprises cefpodoxime proxetil covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a 20 homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have 25 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The

folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and

at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

5 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

 Selection of the amino acids will depend on the physical properties desired. For
10 instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
15 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
20 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
25 synergistic effect between two or more active agents is desired.

 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain

length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to

poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

5 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
10 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
15 carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
20 specific properties to the drug delivery system.

 The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
25 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
30 alimentary tract can affect release.

CW076P

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cefpodoxime proxetil is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the

mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cefpodoxime proxetil conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
5 added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
10 solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

15 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

20 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product
25 precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product,
5 which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and
10 filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cefpodoxime proxetil covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cefpodoxime proxetil is covalently
attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cefpodoxime proxetil is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cefpodoxime proxetil from said composition in a pH-dependent manner.

15 19. A method for protecting cefpodoxime proxetil from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cefpodoxime proxetil from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cefpodoxime proxetil to said polypeptide.

20 21. A method for delivering cefpodoxime proxetil to a patient comprising administering to said patient a composition comprising:

 a polypeptide; and

 cefpodoxime proxetil covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cefpodoxime proxetil is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cefpodoxime proxetil is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 **Abstract**

 A composition comprising a polypeptide and cefpodoxime proxetil covalently attached to the polypeptide. Also provided is a method for delivery of cefpodoxime proxetil to a patient comprising administering to the patient a composition comprising a polypeptide and cefpodoxime proxetil covalently attached to the polypeptide. Also
15 provided is a method for protecting cefpodoxime proxetil from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cefpodoxime proxetil from a composition comprising covalently attaching it to the polypeptide.

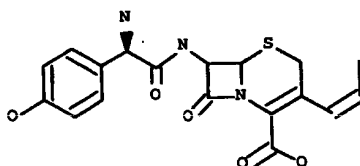
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFPROZIL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cefprozil, as well as methods for protecting and administering cefprozil. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Cefprozil is a known pharmaceutical agent that is used in the treatment of upper
15 respiratory tract infections, otitis media, acute exacerbation of chronic bronchitis, and skin infections. Its chemical name is [6R-[6alpha,7beta(R*)]]-7-[[amino(4-hydroxyphenyl)acetyl]amino-8-oxo-3-(1-propenyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical
25 compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cefprozil) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefprozil to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cefprozil microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cefprozil covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cefprozil preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cefprozil from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cefprozil to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cefprozil is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cefprozil is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cefprozil is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cefprozil is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cefprozil is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cefprozil to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefprozil and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW077P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cefprozil and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cefprozil. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Cefprozil is the subject of GB 2135305 B (1987), based on US Patent Application Serial Number 461833 (1983), herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises cefprozil covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior
10 and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

CW077P

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 25 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

CW077P

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

CW077P

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

CW077P

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cefprozil is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW077P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cefprozil conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW077P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW077P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cefprozil covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cefprozil is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cefprozil is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cefprozil from said composition in a pH-dependent manner.

15 19. A method for protecting cefprozil from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cefprozil from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cefprozil to said polypeptide.

20 21. A method for delivering cefprozil to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cefprozil covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cefprozil is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cefprozil is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cefprozil covalently attached to the polypeptide. Also provided is a method for delivery of cefprozil to a patient comprising administering to the patient a composition comprising a polypeptide and cefprozil covalently attached to the polypeptide. Also provided is a method for protecting cefprozil from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cefprozil from a composition comprising covalently attaching it to the polypeptide.

5

CW078P

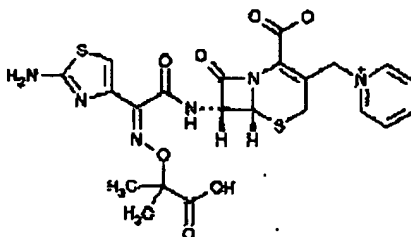
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFTAZIDIME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises
5 a polypeptide that is preferably covalently attached to ceftazidime, as well as methods for
protecting and administering ceftazidime. This novel compound, referred to as a
CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known
effective pharmaceutical agent that is both well studied and occupies a known segment of
the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical
effectiveness.

BACKGROUND OF THE INVENTION

Ceftazidime is a known pharmaceutical agent that is used in the treatment of
bacterial infections. It is both commercially available and readily manufactured using
15 published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
of the original compound; alteration of the release profile of an orally administered
20 product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical
compound may contain one or more of the following: another active pharmaceutical
agent, an adjuvant, or an inhibitor.

CW078P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW078P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (ceftazidime) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ceftazidime to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising ceftazidime microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ceftazidime covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Ceftazidime preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting ceftazidime from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering ceftazidime to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ceftazidime is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, ceftazidime is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ceftazidime is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ceftazidime is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ceftazidime is released from the

CW078P

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching ceftazidime to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ceftazidime and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the
20 active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize ceftazidime and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of ceftazidime. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises ceftazidime covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW078P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

CW078P

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, ceftazidime is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-ceftazidime conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 ceftazidime covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein ceftazidime is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ceftazidime is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ceftazidime from said composition in a pH-dependent manner.

15 19. A method for protecting ceftazidime from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ceftazidime from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ceftazidime to said polypeptide.

20 21. A method for delivering ceftazidime to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
ceftazidime covalently attached to said polypeptide.

25 22. The method of claim 21 wherein ceftazidime is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein ceftazidime is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and ceftazidime covalently attached to the polypeptide. Also provided is a method for delivery of ceftazidime to a patient comprising administering to the patient a composition comprising a polypeptide and
5 ceftazidime covalently attached to the polypeptide. Also provided is a method for protecting ceftazidime from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ceftazidime from a composition comprising covalently attaching it to the polypeptide.

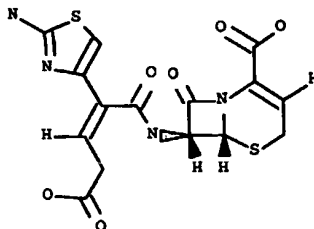
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFTIBUTEN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ceftibuten, as well as methods for protecting and administering ceftibuten. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Ceftibuten is a known pharmaceutical agent that is used in the treatment of
15 bacterial infections. Its chemical name is [6R-[6 α ,7 β (Z)]]-7-[[2-(2-amino-4-thiazolyl)-4-carboxy-1-oxo-2-butenyl]amino]-8-oxo 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW079P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW079P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (ceftibuten) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ceftibuten to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising ceftibuten microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ceftibuten covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Ceftibuten preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW079P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting ceftibuten from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering ceftibuten to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ceftibuten is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, ceftibuten is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ceftibuten is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ceftibuten is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ceftibuten is released from the composition in a sustained release.

CW079P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cefibuten to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefibuten and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular

20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the

25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW079P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize ceftibuten and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of ceftibuten. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Ceftibuten is the subject of EP 136721 B (1993), herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises ceftibuten covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW079P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW079P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW079P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW079P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW079P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, ceftibuten is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW079P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-ceftibuten conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW079P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW079P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW079P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 ceftibuten covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein ceftibuten is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW079P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ceftibuten is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ceftibuten from said composition in a pH-dependent manner.

15 19. A method for protecting ceftibuten from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ceftibuten from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ceftibuten to said polypeptide.

20 21. A method for delivering ceftibuten to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
ceftibuten covalently attached to said polypeptide.

25 22. The method of claim 21 wherein ceftibuten is released from said composition by an enzyme-catalyzed release.

CW079P

23. The method of claim 21 wherein ceftibuten is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW079P

Abstract

A composition comprising a polypeptide and ceftibuten covalently attached to the polypeptide. Also provided is a method for delivery of ceftibuten to a patient comprising administering to the patient a composition comprising a polypeptide and ceftibuten covalently attached to the polypeptide. Also provided is a method for protecting ceftibuten from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ceftibuten from a composition comprising covalently attaching it to the polypeptide.

CW080P

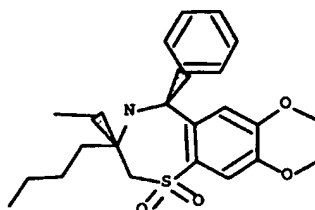
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING A BILE ACID
TRANSPORT INHIBITOR AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises
a polypeptide that is preferably covalently attached to a bile acid transport inhibitor, as
well as methods for protecting and administering a bile acid transport inhibitor. This
novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has
the benefit of taking a known effective pharmaceutical agent that is both well studied and
10 occupies a known segment of the pharmaceutical market, and combining it with a carrier
compound that enhances the usefulness of the pharmaceutical agent without
compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 The bile acid transport inhibitor of the present invention is a known
15 pharmaceutical agent that is used in the treatment of hypercholesterolemia. Its chemical
name is (3R,5R)-rel-3-butyl-3-ethyl-2,3,4,5-tetrahydro-7,8-dimethoxy-5-phenyl-1,4-
benzothiazepine 1,1-dioxide. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability
of the original compound; alteration of the release profile of an orally administered
product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical
compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW080P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (The bile acid transport inhibitor of the present invention) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching the bile acid transport inhibitor of the present invention to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising the bile acid transport inhibitor of the present invention microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and the bile acid transport inhibitor of the present invention covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW080P

The bile acid transport inhibitor of the present invention preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting the bile acid transport inhibitor of the present invention from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering the bile acid transport inhibitor of the present invention to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, the bile acid transport inhibitor of the present invention is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, the bile acid transport inhibitor of the present invention is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the

CW080P

composition further comprises a microencapsulating agent and the bile acid transport inhibitor of the present invention is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, the bile acid transport inhibitor of the present invention is released from the composition by a pH-dependent
5 unfolding of the polypeptide. In another preferred embodiment, the bile acid transport inhibitor of the present invention is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier
10 peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching the bile acid transport inhibitor of the present invention to a side chain of an
15 amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
(NCA).

20 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, the bile acid transport inhibitor of the present invention and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a
25 hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an
30 ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a

CW080P

pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

- 5 The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- The present invention provides several benefits for active agent delivery. First,
10 the invention can stabilize the bile acid transport inhibitor of the present invention and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of the bile acid transport inhibitor of the present invention. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also
15 allows targeted delivery of active agents to specific sites of action.

The bile acid transport inhibitor of the present invention is the subject of WO 96/5188 (1996), based on US application 288527 (1994), herein incorporated by reference, which describes how to make that drug.

- The composition of the invention comprises the bile acid transport inhibitor of the
20 present invention covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more
25 synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and

turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

- 5 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the
- 10 protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

- The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
- 15 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
- 20 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
- 25 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is

CW080P

a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

- 5 Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different
10 than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 15 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
20 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine,
25 aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

CW080P

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to

CW080P

poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

5 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
10 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
15 carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
20 specific properties to the drug delivery system.

 The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
25 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
30 alimentary tract can affect release.

CW080P

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
5 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
10 short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

15 The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex,
20 PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
25 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,

CW080P

sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
5 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
10 absorption of the peptides.

Preferably, the resultant peptide-bile acid transport inhibitor conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids
15 and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be
20 stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
25 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours

CW080P

at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for

CW080P

several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
5 the mixture refluxed under a nitrogen atmosphere until the mixture becomes
homogenous. The solution can be poured into heptane to precipitate the NCA product,
which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
10 a primary amine can be added to the solution until it becomes viscous (typically
overnight). The product can be isolated from the solution by pouring it into water and
filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments,
the invention is nevertheless not intended to be limited to the details shown. Rather,
15 various modifications may be made in the details within the scope and range of
equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 a bile acid transport inhibitor covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
15 one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein the bile acid transport inhibitor is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
10. The composition of claim 9 wherein said microencapsulating agent is
20 selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein the bile acid transport inhibitor is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing the bile acid transport inhibitor from said composition in a pH-dependent manner.

15 19. A method for protecting a bile acid transport inhibitor from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of a bile acid transport inhibitor from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching the bile acid transport inhibitor to said polypeptide.

20 21. A method for delivering a bile acid transport inhibitor to a patient comprising administering to said patient a composition comprising:

 a polypeptide; and

 a bile acid transport inhibitor covalently attached to said polypeptide.

25 22. The method of claim 21 wherein the bile acid transport inhibitor is released from said composition by an enzyme-catalyzed release.

CW080P

23. The method of claim 21 wherein the bile acid transport inhibitor is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 **Abstract**

 A composition comprising a polypeptide and a bile acid transport inhibitor covalently attached to the polypeptide. Also provided is a method for delivery of a bile acid transport inhibitor to a patient comprising administering to the patient a composition comprising a polypeptide and a bile acid transport inhibitor covalently attached to the
15 polypeptide. Also provided is a method for protecting a bile acid transport inhibitor from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of a bile acid transport inhibitor from a composition comprising covalently attaching it to the polypeptide.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW081P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW081P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cefuroxime axetil) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefuroxime axetil to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cefuroxime axetil microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cefuroxime axetil covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cefuroxime axetil preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active

CW081P

agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet
5 another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino
10 acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In
15 another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cefuroxime axetil from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cefuroxime axetil to a
20 patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cefuroxime axetil is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cefuroxime axetil is released in a time-dependent manner based on the pharmacokinetics
25 of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cefuroxime axetil is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cefuroxime axetil is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cefuroxime axetil is

released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching cefuroxime axetil to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefuroxime axetil and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cefuroxime axetil and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cefuroxime axetil. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Cefuroxime axetil is the subject of GB 1571683 (1980), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises cefuroxime axetil covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW081P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

- 5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
- 10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
- 15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

- Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
- 20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
- 25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border

10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For

20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW081P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cefuroxime axetil is covalently attached to the polypeptide via the amido group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-cefuroxime axetil conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW081P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cefuroxime axetil covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cefuroxime axetil is covalently attached to
a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cefuroxime axetil is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cefuroxime axetil from said composition in a pH-dependent manner.

15 19. A method for protecting cefuroxime axetil from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cefuroxime axetil from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cefuroxime axetil to said polypeptide.

20 21. A method for delivering cefuroxime axetil to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cefuroxime axetil covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cefuroxime axetil is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cefuroxime axetil is released from said composition by a pH-dependent unfolding of said polypeptide.
24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.
- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cefuroxime axetil covalently attached to the polypeptide. Also provided is a method for delivery of cefuroxime axetil to a patient comprising administering to the patient a composition comprising a polypeptide and cefuroxime axetil covalently attached to the polypeptide. Also provided is a method for protecting cefuroxime axetil from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cefuroxime axetil from a composition comprising covalently attaching it to the polypeptide.

CW082P

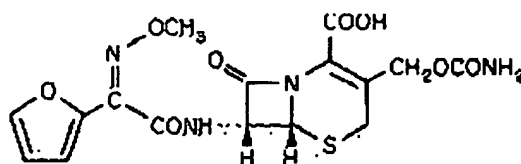
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEFUROXIME AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cefuroxime, as well as methods for protecting and administering cefuroxime. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Cefuroxime is a known pharmaceutical agent that is used in the treatment of
15 bacterial infection. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

CW082P

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

CW082P

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (cefuroxime) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cefuroxime to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the
10 polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This
15 enzymatic action introduces a second order sustained release mechanism.

 Alternatively, the present invention provides a pharmaceutical composition comprising cefuroxime microencapsulated by a polypeptide.

 The invention provides a composition comprising a polypeptide and cefuroxime covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,
20 (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 Cefuroxime preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting cefuroxime from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering cefuroxime to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the
20 polypeptide. In a preferred embodiment, cefuroxime is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cefuroxime is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cefuroxime is released from the composition by dissolution
25 of the microencapsulating agent. In another preferred embodiment, cefuroxime is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cefuroxime is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant

from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cefuroxime to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
- from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cefuroxime and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize cefuroxime and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cefuroxime.

5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises cefuroxime covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one

10 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have

15 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains

20 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

25 acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
 5 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
 15 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
 20 conceivably have a loading of 58%, although this may not be entirely practical.

CW082P

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

CW082P

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cefuroxime is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

CW082P

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized

5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,

10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is

15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-cefuroxime conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

- The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
- 15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
- 20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
- 25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- 20 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cefuroxime covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cefuroxime is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW082P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cefuroxime is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cefuroxime from said composition in a pH-dependent manner.

15 19. A method for protecting cefuroxime from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cefuroxime from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cefuroxime to said polypeptide.

20 21. A method for delivering cefuroxime to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cefuroxime covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cefuroxime is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cefuroxime is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cefuroxime covalently attached to the polypeptide. Also provided is a method for delivery of cefuroxime to a patient comprising administering to the patient a composition comprising a polypeptide and cefuroxime covalently attached to the polypeptide. Also provided is a method for protecting cefuroxime from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cefuroxime from a composition comprising covalently attaching it to the polypeptide.

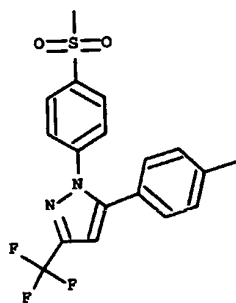
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CELECOXIB AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to celecoxib, as well as methods for protecting and administering celecoxib. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Celecoxib is a known pharmaceutical agent that is used in the treatment of osteo-
15 and rheumatoid arthritis. Its chemical name is 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW083P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous 15 pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.

20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide 25 linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable 30

CW083P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent
10 (celecoxib) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching celecoxib to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising celecoxib microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and celecoxib covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW083P

Celecoxib preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting celecoxib from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering celecoxib to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, celecoxib is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, celecoxib is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and celecoxib is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, celecoxib is released

CW083P

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, celecoxib is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching celecoxib to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, celecoxib and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize celecoxib and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of celecoxib. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also
10 allows targeted delivery of active agents to specific sites of action.

Celecoxib is the subject of U.S. Patent Numbers 5,466,823, 5,563,165, 5,760,068 and 5,972,986, herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises celecoxib covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 25 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
 5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
 10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
 15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
 20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

CW083P

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW083P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-celecoxib conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW083P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 celecoxib covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein celecoxib is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein celecoxib is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing celecoxib from said composition in a pH-dependent manner.

15 19. A method for protecting celecoxib from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of celecoxib from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching celecoxib to said polypeptide.

20 21. A method for delivering celecoxib to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
celecoxib covalently attached to said polypeptide.

25 22. The method of claim 21 wherein celecoxib is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein celecoxib is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

 A composition comprising a polypeptide and celecoxib covalently attached to the polypeptide. Also provided is a method for delivery of celecoxib to a patient comprising administering to the patient a composition comprising a polypeptide and celecoxib covalently attached to the polypeptide. Also provided is a method for protecting
15 celecoxib from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of celecoxib from a composition comprising covalently attaching it to the polypeptide.

CW084P

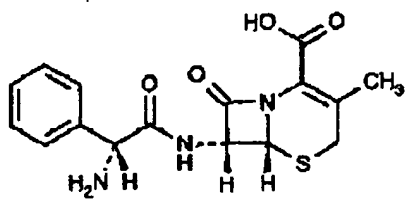
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CEPHALEXIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cephalixin, as well as methods for protecting and administering cephalixin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Cephalexin is a known pharmaceutical agent that is used in the treatment of
15 bacterial infection. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW084P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some
5 technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically
10 require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the
15 gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The
20 released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that
25 incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly
30 or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW084P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cephalexin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cephalexin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cephalexin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cephalexin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cephalexin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW084P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cephalexin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cephalexin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cephalexin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cephalexin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cephalexin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cephalexin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cephalexin is released from the composition in a sustained

release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cephalixin to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cephalixin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW084P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cephalexin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cephalexin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises cephalexin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW084P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

25

CW084P

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW084P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

CW084P

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, cephalexin is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

CW084P

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cephalexin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW084P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cephalexin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cephalexin is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW084P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cephalixin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cephalixin from said composition in a pH-dependent manner.

15 19. A method for protecting cephalixin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cephalixin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cephalixin to said polypeptide.

20 21. A method for delivering cephalixin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cephalixin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cephalixin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cephalixin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cephalixin covalently attached to the polypeptide. Also provided is a method for delivery of cephalixin to a patient comprising administering to the patient a composition comprising a polypeptide and cephalixin covalently attached to the polypeptide. Also provided is a method for
5 protecting cephalixin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cephalixin from a composition comprising covalently attaching it to the polypeptide.

CW085P

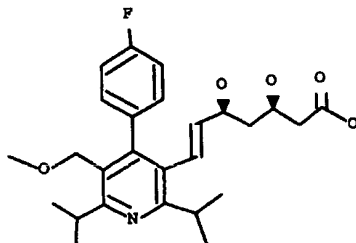
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CERIVASTATIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cerivastatin, as well as methods for protecting and administering cerivastatin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Cerivastatin is a known pharmaceutical agent that is used in the treatment of
15 cholesterolemia. Its chemical name is [S-[R*,S*-(E)]]-7-[4-(4-fluorophenyl)-5-(methoxymethyl)-2,6-bis (1-methylethyl)-3-pyridinyl]-3,5-dihydroxy-6-heptenoic acid. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW085P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.

20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW085P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
10 (cerivastatin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cerivastatin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising cerivastatin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cerivastatin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW085P

Cerivastatin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cerivastatin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cerivastatin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cerivastatin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cerivastatin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cerivastatin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cerivastatin is

CW085P

released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cerivastatin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant
5 from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
10 comprises the steps of:

- (a) attaching cerivastatin to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
15 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cerivastatin and a second active agent can be copolymerized in step (c). In another
20 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
25 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000,
5 incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize cerivastatin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cerivastatin.
10 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Cerivastatin is the subject of U.S. Patent Number 5,177,080, herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises cerivastatin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
20 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
25 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW085P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW085P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW085P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, cerivastatin is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW085P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cerivastatin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW085P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to
5 0°C. The solution can then be treated with diisopropylcarbodiimide and
hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be
stirred for several hours at room temperature, the urea by-product filtered off, the product
precipitated out in ether and purified using gel permeation chromatography (GPC) or
dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
followed by the amine bioactive agent. The reaction can then be stirred for several hours
at room temperature, the urea by-product filtered off, and the product precipitated out in
15 ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene
produces a chloroformate, which when reacted with the N-terminus of the peptide
produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with
20 triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
added slowly and the solution stirred at room temperature for several hours. The product
is then precipitated out in ether. The crude product is suitably deprotected and purified
using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of
25 other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
solvents such as chloroform. Examples of other activating agents include
dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cerivastatin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
15 one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cerivastatin is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
10. The composition of claim 9 wherein said microencapsulating agent is
20 selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cerivastatin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cerivastatin from said composition in a pH-dependent manner.

15 19. A method for protecting cerivastatin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cerivastatin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cerivastatin to said polypeptide.

20 21. A method for delivering cerivastatin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cerivastatin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cerivastatin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cerivastatin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 **Abstract**

A composition comprising a polypeptide and cerivastatin covalently attached to the polypeptide. Also provided is a method for delivery of cerivastatin to a patient comprising administering to the patient a composition comprising a polypeptide and cerivastatin covalently attached to the polypeptide. Also provided is a method for
15 protecting cerivastatin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cerivastatin from a composition comprising covalently attaching it to the polypeptide.

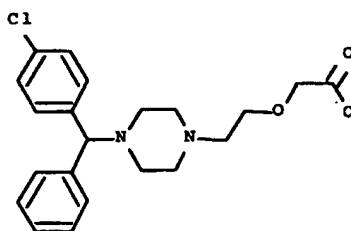
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CETIRIZINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cetirizine, as well as methods for protecting and administering cetirizine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Cetirizine is a known pharmaceutical agent that is used in the treatment of allergic rhinitis. Its chemical name is [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW086P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW086P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW086P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cetirizine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cetirizine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cetirizine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cetirizine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cetirizine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW086P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cetirizine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cetirizine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cetirizine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cetirizine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cetirizine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cetirizine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cetirizine is released from the composition in a sustained release.

CW086P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cetirizine to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cetirizine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cetirizine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cetirizine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Cetirizine is the subject of U.S. Patent Number 4,525,358, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises cetirizine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

CW086P

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

- 5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
- 10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
- 15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

- Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
- 20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
- 25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW086P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

20

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW086P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cetirizine is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-cetirizine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cetirizine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cetirizine is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW086P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.
- 5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein cetirizine is conformationally protected by folding of said polypeptide about said active agent.
18. The composition of claim 1 wherein said polypeptide is capable of releasing cetirizine from said composition in a pH-dependent manner.
- 15 19. A method for protecting cetirizine from degradation comprising covalently attaching said active agent to a polypeptide.
20. A method for controlling release of cetirizine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cetirizine to said polypeptide.
- 20 21. A method for delivering cetirizine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cetirizine covalently attached to said polypeptide.
22. The method of claim 21 wherein cetirizine is released from said composition
25 by an enzyme-catalyzed release.

23. The method of claim 21 wherein cetirizine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW086P

Abstract

A composition comprising a polypeptide and cetirizine covalently attached to the polypeptide. Also provided is a method for delivery of cetirizine to a patient comprising administering to the patient a composition comprising a polypeptide and cetirizine covalently attached to the polypeptide. Also provided is a method for protecting cetirizine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cetirizine from a composition comprising covalently attaching it to the polypeptide.

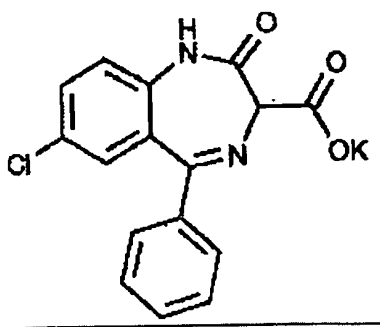
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CHLORAZEPATE DEPOT AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to chlorazepate depot, as well as methods for protecting and administering chlorazepate depot. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Chlorazepate depot is a known pharmaceutical agent that is used in the treatment
15 of anxiety disorders. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

CW087P

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble
5 microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.

20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW087P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
10 (chlorazepate depot) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching chlorazepate depot to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the
15 stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

20 Alternatively, the present invention provides a pharmaceutical composition comprising chlorazepate depot microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and chlorazepate depot covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,
25 (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Chlorazepate depot preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting chlorazepate depot from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering chlorazepate depot to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, chlorazepate depot is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, chlorazepate depot is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and chlorazepate depot is released from the composition by dissolution of the microencapsulating agent. In another preferred

CW087P

embodiment, chlorazepate depot is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, chlorazepate depot is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching chlorazepate depot to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, chlorazepate depot and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize chlorazepate depot and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of chlorazepate depot. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises chlorazepate depot covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW087P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW087P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, chlorazepate depot is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-chlorazepate depot conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW087P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 chlorazepate depot covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein chlorazepate depot is covalently attached
to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein chlorazepate depot is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing chlorazepate depot from said composition in a pH-dependent manner.

15 19. A method for protecting chlorazepate depot from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of chlorazepate depot from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching chlorazepate depot to said polypeptide.

20 21. A method for delivering chlorazepate depot to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
chlorazepate depot covalently attached to said polypeptide.

25 22. The method of claim 21 wherein chlorazepate depot is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein chlorazepate depot is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and chlorazepate depot covalently attached to the polypeptide. Also provided is a method for delivery of chlorazepate depot to a patient comprising administering to the patient a composition comprising a

5 polypeptide and chlorazepate depot covalently attached to the polypeptide. Also provided is a method for protecting chlorazepate depot from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of chlorazepate depot from a composition comprising covalently attaching it to the polypeptide.

CW088P

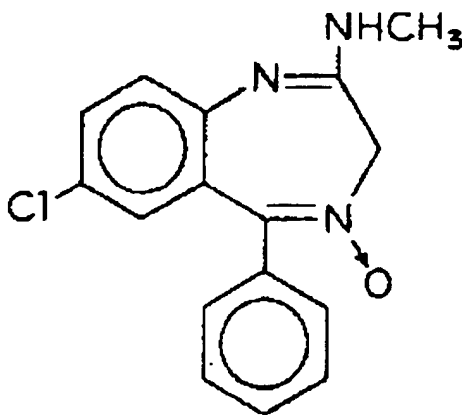
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
CHLORDIAZEPOXIDE AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to chlordiazepoxide, as well as methods for protecting and administering chlordiazepoxide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Chlordiazepoxide is a known pharmaceutical agent that is used in the treatment of anxiety and tension. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;

CW088P

and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

CW088P

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

CW088P

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (chlordiazepoxide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching chlordiazepoxide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising chlordiazepoxide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and chlordiazepoxide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic

CW088P

amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Chlordiazepoxide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a
5 carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-
10 terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
15 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
20 composition in a pH-dependent manner.

The invention also provides a method for protecting chlordiazepoxide from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering chlordiazepoxide to a patient, the patient being a human or a non-human animal, comprising administering to the
25 patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, chlordiazepoxide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, chlordiazepoxide is released in a time-dependent manner based on the pharmacokinetics

CW088P

of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and chlordiazepoxide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, chlordiazepoxide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, chlordiazepoxide is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching chlordiazepoxide to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, chlordiazepoxide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the

CW088P

glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

- 5 The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- 10 The present invention provides several benefits for active agent delivery. First, the invention can stabilize chlordiazepoxide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of chlordiazepoxide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

- 15 The composition of the invention comprises chlordiazepoxide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
20 more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
25 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW088P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW088P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW088P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW088P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, chlordiazepoxide is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW088P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-chlordiazepoxide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW088P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW088P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 chlordiazepoxide covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein chlordiazepoxide is covalently attached to
a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein chlordiazepoxide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing chlordiazepoxide from said composition in a pH-dependent manner.

15 19. A method for protecting chlordiazepoxide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of chlordiazepoxide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching chlordiazepoxide to said polypeptide.

20 21. A method for delivering chlordiazepoxide to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
chlordiazepoxide covalently attached to said polypeptide.

25 22. The method of claim 21 wherein chlordiazepoxide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein chlordiazepoxide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

 A composition comprising a polypeptide and chlordiazepoxide covalently attached to the polypeptide. Also provided is a method for delivery of chlordiazepoxide to a patient comprising administering to the patient a composition comprising a polypeptide and chlordiazepoxide covalently attached to the polypeptide. Also provided
15 is a method for protecting chlordiazepoxide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of chlordiazepoxide from a composition comprising covalently attaching it to the polypeptide.

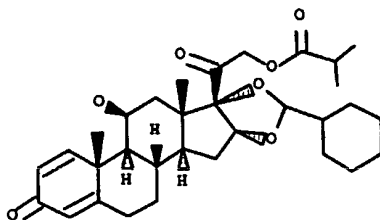
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
CICLESONIDE AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ciclesonide, as well as methods for protecting and administering ciclesonide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Ciclesonide is a known pharmaceutical agent that is used in the treatment of asthma. Its chemical name is [11beta,16alpha (R)]-16,17-
15 [(cyclohexylmethylene)bis(oxy)]-11-hydroxy-21-(2-methyl-1-oxopropoxy)-pregna-1,4-diene-3,20-dione. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

CW089P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW089P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW089P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (ciclesonide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ciclesonide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising ciclesonide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ciclesonide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Ciclesonide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW089P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting ciclesonide from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering ciclesonide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ciclesonide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, ciclesonide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ciclesonide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ciclesonide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ciclesonide is released from the composition in a

CW089P

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching ciclosonide to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ciclosonide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW089P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize ciclesonide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of ciclesonide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Ciclesonide is the subject of GB 2247680 B (1994), based on US Application Number 578942 (1990), herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises ciclesonide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

CW089P

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
 5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
 10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
 15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
 20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

CW089P

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, ciclesonide is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW089P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-ciclesonide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 ciclesonide covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein ciclesonide is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ciclesonide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ciclesonide from said composition in a pH-dependent manner.

15 19. A method for protecting ciclesonide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ciclesonide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ciclesonide to said polypeptide.

20 21. A method for delivering ciclesonide to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
ciclesonide covalently attached to said polypeptide.

25 22. The method of claim 21 wherein ciclesonide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein ciclesonide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and ciclesonide covalently attached to the polypeptide. Also provided is a method for delivery of ciclesonide to a patient comprising administering to the patient a composition comprising a polypeptide and ciclesonide covalently attached to the polypeptide. Also provided is a method for protecting ciclesonide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ciclesonide from a composition comprising covalently attaching it to the polypeptide.

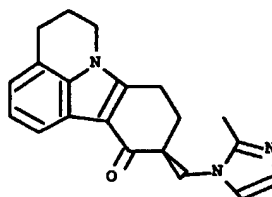
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CILANSETRON AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cilansetron, as well as methods for protecting and administering cilansetron. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Cilansetron is a known pharmaceutical agent that is used in the treatment of
15 irritable bowel syndrome. Its chemical name is (R)-5,6,9,10-tetrahydro-10-[(2-methyl-
1H-imidazol-1-yl)methyl]-4H-pyrido[3,2,1-jk]carbazol-11(8H)-one. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW090P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW090P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW090P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cilansetron) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cilansetron to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cilansetron microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cilansetron covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cilansetron preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cilansetron from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cilansetron to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cilansetron is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cilansetron is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cilansetron is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cilansetron is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cilansetron is released from the composition in a

CW090P

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cilansetron to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cilansetron and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW090P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cilansetron and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cilansetron. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Cilansetron is the subject of EP 297651 (1989), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises cilansetron covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

- 5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
- 10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
- 15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

- Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
- 20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
- 25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
25 short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW090P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-cilansetron conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW090P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cilansetron covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cilansetron is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cilansetron is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cilansetron from said composition in a pH-dependent manner.

15 19. A method for protecting cilansetron from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cilansetron from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cilansetron to said polypeptide.

20 21. A method for delivering cilansetron to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cilansetron covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cilansetron is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cilansetron is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cilansetron covalently attached to the polypeptide. Also provided is a method for delivery of cilansetron to a patient comprising administering to the patient a composition comprising a polypeptide and
5 cilansetron covalently attached to the polypeptide. Also provided is a method for protecting cilansetron from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cilansetron from a composition comprising covalently attaching it to the polypeptide.

CW091P

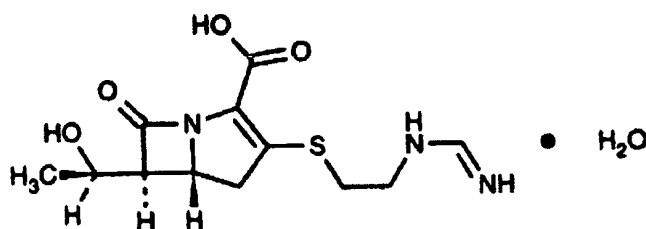
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
CILASTATIN AND IMPENEM AND METHODS OF
MAKING AND USING SAME**

5 FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cilastatin and imipenem, as well as methods for protecting and administering cilastatin and imipenem. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Cilastatin and imipenem are known pharmaceutical agents that are used together in the treatment of bacterial infections. Cilastatin has no antibacterial activity, but increases the effectiveness of imipenem. Each is commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. The structure of imipenem is:



20

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

25

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW091P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent (cilastatin
10 and imipenem) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cilastatin and imipenem to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach,
15 through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising cilastatin and imipenem microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and cilastatin and imipenem covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,
25 (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cilastatin and imipenem preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached
5 to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a
10 microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet,
15 an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cilastatin and imipenem from
20 degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cilastatin and imipenem to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cilastatin and imipenem are
25 released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cilastatin and imipenem are released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cilastatin and imipenem are released from the composition by dissolution of the microencapsulating

CW091P

agent. In another preferred embodiment, cilastatin and imipenem are released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cilastatin and imipenem are released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an
5 adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
10 comprises the steps of:

- (a) attaching cilastatin and imipenem to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
15 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cilastatin and imipenem and a second active agent can be copolymerized in step
20 (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is
25 attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid
30 functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize cilastatin and imipenem and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cilastatin and imipenem. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises cilastatin and imipenem covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

CW091P

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, imipenem is covalently attached to the polypeptide via the carboxylic acid. Ciliastatin can be attached via any free alcohol, acid, or amine group, or can be attached via a linker.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW091P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cilastatin and imipenem conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW091P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cilastatin and imipenem covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cilastatin and imipenem are covalently
attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.
- 5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein cilastatin and imipenem are conformationally protected by folding of said polypeptide about said active agent.
18. The composition of claim 1 wherein said polypeptide is capable of releasing cilastatin and imipenem from said composition in a pH-dependent manner.
- 15 19. A method for protecting cilastatin and imipenem from degradation comprising covalently attaching said active agent to a polypeptide.
- 20 20. A method for controlling release of cilastatin and imipenem from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cilastatin and imipenem to said polypeptide.
- 20 21. A method for delivering cilastatin and imipenem to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cilastatin and imipenem covalently attached to said polypeptide.
- 25 22. The method of claim 21 wherein cilastatin and imipenem are released from said composition by an enzyme-catalyzed release.

CW091P

23. The method of claim 21 wherein cilastatin and imipenem are released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cilastatin and imipenem covalently attached to the polypeptide. Also provided is a method for delivery of cilastatin and imipenem to a patient comprising administering to the patient a composition comprising a polypeptide and cilastatin and imipenem covalently attached to the polypeptide. Also provided is a method for protecting cilastatin and imipenem from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cilastatin and imipenem from a composition comprising covalently attaching it to the polypeptide.

CW092P

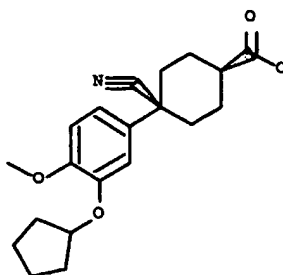
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CILOMILAST AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cilomilast, as well as methods for protecting and administering cilomilast. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Cilomilast is a known pharmaceutical agent that is used in the treatment of
15 asthma. Its chemical name is cis-4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl] cyclohexanecarboxylic acid. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW092P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cilomilast) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cilomilast to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cilomilast microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cilomilast covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cilomilast preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cilomilast from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cilomilast to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cilomilast is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cilomilast is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cilomilast is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cilomilast is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cilomilast is released from the composition in a sustained release.

CW092P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cilomilast to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cilomilast and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First,
5 the invention can stabilize cilomilast and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cilomilast. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Cilomilast is the subject of WO 93/19749 (1993), based on priority US application 862030 (1992), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises cilomilast covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
15 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have
20 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior
10 and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

CW092P

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
 5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
 10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
 15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
 20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

CW092P

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cilomilast is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW092P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-cilomilast conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW092P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cilomilast covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cilomilast is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.
- 5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein cilomilast is conformationally protected by folding of said polypeptide about said active agent.
18. The composition of claim 1 wherein said polypeptide is capable of releasing cilomilast from said composition in a pH-dependent manner.
- 15 19. A method for protecting cilomilast from degradation comprising covalently attaching said active agent to a polypeptide.
20. A method for controlling release of cilomilast from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cilomilast to said polypeptide.
- 20 21. A method for delivering cilomilast to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cilomilast covalently attached to said polypeptide.
22. The method of claim 21 wherein cilomilast is released from said composition
25 by an enzyme-catalyzed release.

23. The method of claim 21 wherein cilomilast is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cilomilast covalently attached to the polypeptide. Also provided is a method for delivery of cilomilast to a patient comprising administering to the patient a composition comprising a polypeptide and cilomilast covalently attached to the polypeptide. Also provided is a method for protecting
5 cilomilast from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cilomilast from a composition comprising covalently attaching it to the polypeptide.

CW093P

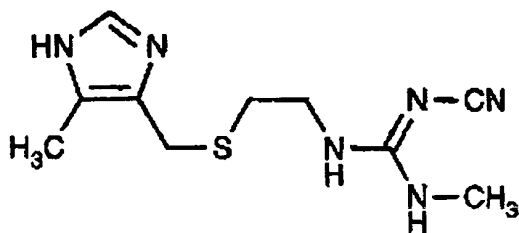
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CIMETIDINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cimetidine, as well as methods for protecting and administering cimetidine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Cimetidine is a known pharmaceutical agent that is used in the treatment of
15 duodenal ulcer. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW093P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW093P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cimetidine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cimetidine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cimetidine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cimetidine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cimetidine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cimetidine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cimetidine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cimetidine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cimetidine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cimetidine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cimetidine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cimetidine is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cimetidine to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cimetidine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First,
5 the invention can stabilize cimetidine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cimetidine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises cimetidine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, cimetidine is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

CW093P

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
5 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the
10 transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active
15 agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the
20 catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cimetidine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

25 Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW093P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW093P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments,
the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW093P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cimetidine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cimetidine is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW093P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cimetidine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cimetidine from said composition in a pH-dependent manner.

15 19. A method for protecting cimetidine from degradation comprising covalently attaching said active agent to a polypeptide.

20 20. A method for controlling release of cimetidine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cimetidine to said polypeptide.

21. A method for delivering cimetidine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cimetidine covalently attached to said polypeptide.

22. The method of claim 21 wherein cimetidine is released from said composition
25 by an enzyme-catalyzed release.

23. The method of claim 21 wherein cimetidine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cimetidine covalently attached to the polypeptide. Also provided is a method for delivery of cimetidine to a patient comprising administering to the patient a composition comprising a polypeptide and cimetidine covalently attached to the polypeptide. Also provided is a method for protecting
5 cimetidine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cimetidine from a composition comprising covalently attaching it to the polypeptide.

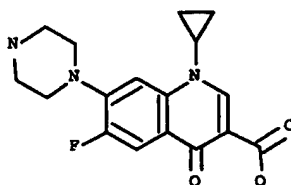
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CIPROFLOXACIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ciprofloxacin, as well as methods for protecting and administering ciprofloxacin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Ciprofloxacin is a known pharmaceutical agent that is used in the treatment of
15 bacterial infection. Its chemical name is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW094P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW094P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (ciprofloxacin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ciprofloxacin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising ciprofloxacin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ciprofloxacin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Ciprofloxacin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting ciprofloxacin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering ciprofloxacin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ciprofloxacin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, ciprofloxacin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ciprofloxacin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ciprofloxacin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ciprofloxacin is released from the

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching ciprofloxacin to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ciprofloxacin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize ciprofloxacin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of ciprofloxacin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Ciprofloxacin is the subject of U.S. Patent Numbers 4,670,444 and 5,286,754, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises ciprofloxacin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

- 5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
- 10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
- 15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

- Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
- 20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
- 25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW094P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

CW094P

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, ciprofloxacin is covalently attached to the polypeptide via the carboxylic acid.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-ciprofloxacin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW094P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 ciprofloxacin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein ciprofloxacin is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.
- 5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein ciprofloxacin is conformationally protected by folding of said polypeptide about said active agent.
18. The composition of claim 1 wherein said polypeptide is capable of releasing ciprofloxacin from said composition in a pH-dependent manner.
- 15 19. A method for protecting ciprofloxacin from degradation comprising covalently attaching said active agent to a polypeptide.
20. A method for controlling release of ciprofloxacin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ciprofloxacin to said polypeptide.
- 20 21. A method for delivering ciprofloxacin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
ciprofloxacin covalently attached to said polypeptide.
22. The method of claim 21 wherein ciprofloxacin is released from said
25 composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein ciprofloxacin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and ciprofloxacin covalently attached to the polypeptide. Also provided is a method for delivery of ciprofloxacin to a patient comprising administering to the patient a composition comprising a polypeptide and ciprofloxacin covalently attached to the polypeptide. Also provided is a method for protecting ciprofloxacin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ciprofloxacin from a composition comprising covalently attaching it to the polypeptide.

CW095P

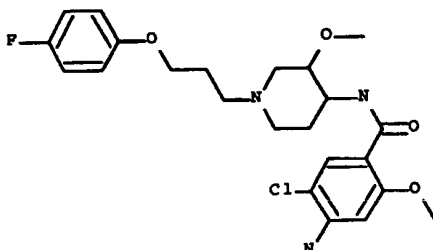
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CISAPRIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cisapride, as well as methods for protecting and administering cisapride. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Cisapride is a known pharmaceutical agent that is used in the treatment of
15 gastrointestinal motility disease. Its chemical name is cis-4-amino-5-chloro-N-[1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-piperidinyl]-2-methoxybenzamide. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW095P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW095P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW095P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cisapride) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cisapride to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cisapride microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cisapride covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cisapride preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cisapride from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cisapride to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cisapride is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cisapride is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cisapride is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cisapride is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cisapride is released from the composition in a sustained release.

CW095P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cisapride to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cisapride and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW095P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cisapride and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cisapride. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Cisapride is the subject of U.S. Patent Number 4,962,115, herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises cisapride covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW095P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW095P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, cisapride is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cisapride conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW095P

Acid/N-terminus conjugation

- An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

- The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cisapride covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cisapride is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW095P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cisapride is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cisapride from said composition in a pH-dependent manner.

15 19. A method for protecting cisapride from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cisapride from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cisapride to said polypeptide.

20 21. A method for delivering cisapride to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cisapride covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cisapride is released from said composition by an enzyme-catalyzed release.

CW095P

23. The method of claim 21 wherein cisapride is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cisapride covalently attached to the polypeptide. Also provided is a method for delivery of cisapride to a patient comprising administering to the patient a composition comprising a polypeptide and cisapride covalently attached to the polypeptide. Also provided is a method for protecting
5 cisapride from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cisapride from a composition comprising covalently attaching it to the polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CISATRACURIUM BESYLATE AND METHODS OF MAKING AND USING SAME

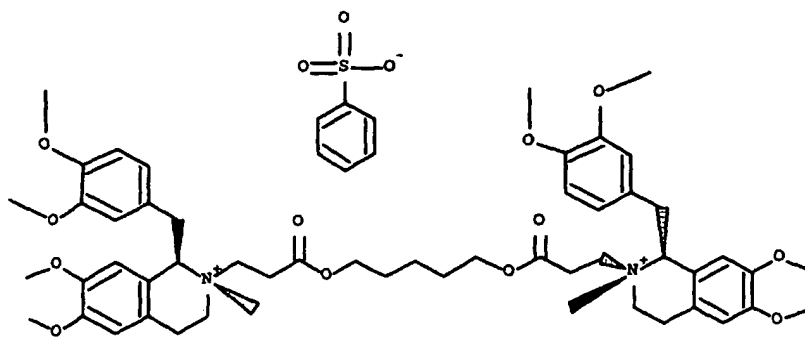
5 FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cisatracurium besylate, as well as methods for protecting and administering cisatracurium besylate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of

10 taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Cisatracurium besylate is a known pharmaceutical agent that is used as a neuromuscular blocker in surgery. Its chemical name is [1R-[1alpha,2alpha(1'R*,2'R*)]]-2,2'-[1,5-pentanediy]bis[oxy(3-oxoo-3,1-propanediyl)]]bis[1-[(3,4-dimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-isoquinolinium]. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;

CW096P

and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

CW096P

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cisatracurium besylate) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cisatracurium besylate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cisatracurium besylate microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cisatracurium besylate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or

CW096P

(vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cisatracurium besylate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cisatracurium besylate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cisatracurium besylate to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cisatracurium besylate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cisatracurium besylate is released in a time-dependent manner based on the

CW096P

pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cisatracurium besylate is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cisatracurium besylate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cisatracurium besylate is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cisatracurium besylate to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cisatracurium besylate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the

CW096P

glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

- 5 The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

- 10 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cisatracurium besylate and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cisatracurium besylate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.
- 15

Cisatracurium besylate is the subject of U.S. Patent Number 5,453,510 and WO 92/965 (1992), herein incorporated by reference, which describes how to make that drug.

- 20 The composition of the invention comprises cisatracurium besylate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

- 25 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The

folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and

CW096P

at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

5 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

 Selection of the amino acids will depend on the physical properties desired. For
10 instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
15 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
20 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
25 synergistic effect between two or more active agents is desired.

 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain

CW096P

length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to

CW096P

poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

5 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
10 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using
15 any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
20 specific properties to the drug delivery system.

 The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
25 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
30 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,

sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
5 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
10 absorption of the peptides.

Preferably, the resultant peptide-cisatracurium besylate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

15 **Acid/N-terminus conjugation**

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product
20 precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
25 followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

CW096P

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with
5 triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of
10 other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

15 Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered,
20 dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for
25 several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product,
5 which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and
10 filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cisatracurium besylate covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cisatracurium besylate is covalently
attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW096P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cisatracurium besylate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cisatracurium besylate from said composition in a pH-dependent manner.

15 19. A method for protecting cisatracurium besylate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cisatracurium besylate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cisatracurium besylate to said polypeptide.

20 21. A method for delivering cisatracurium besylate to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

cisatracurium besylate covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cisatracurium besylate is released from said composition by an enzyme-catalyzed release.

CW096P

23. The method of claim 21 wherein cisatracurium besylate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

 A composition comprising a polypeptide and cisatracurium besylate covalently attached to the polypeptide. Also provided is a method for delivery of cisatracurium besylate to a patient comprising administering to the patient a composition comprising a polypeptide and cisatracurium besylate covalently attached to the polypeptide. Also
15 provided is a method for protecting cisatracurium besylate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cisatracurium besylate from a composition comprising covalently attaching it to the polypeptide.

CW097P

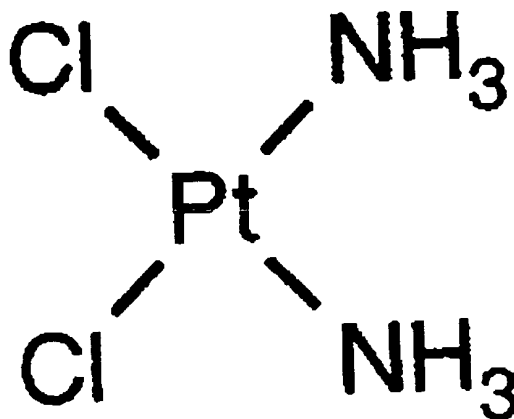
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CISPLATIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cisplatin, as well as methods for protecting and administering cisplatin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Cisplatin is a known pharmaceutical agent that is used in the treatment of bladder and ovarian carcinoma. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;

CW097P

and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

CW097P

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

CW097P

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cisplatin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cisplatin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cisplatin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cisplatin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

CW097P

heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cisplatin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a
5 carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-
10 terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
15 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
20 composition in a pH-dependent manner.

The invention also provides a method for protecting cisplatin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cisplatin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a
25 composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cisplatin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cisplatin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release.

CW097P

In another preferred embodiment, the composition further comprises a microencapsulating agent and cisplatin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cisplatin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cisplatin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

10 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cisplatin to a side chain of an amino acid to form an active agent/amino acid complex;
- 15 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cisplatin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

CW097P

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize cisplatin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cisplatin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Cisplatin is the subject of U.S. Patent Number 5,562,925, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises cisplatin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW097P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW097P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW097P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW097P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, cisplatin is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW097P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cisplatin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW097P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW097P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW097P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cisplatin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cisplatin is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW097P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.
- 5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein cisplatin is conformationally protected by folding of said polypeptide about said active agent.
18. The composition of claim 1 wherein said polypeptide is capable of releasing cisplatin from said composition in a pH-dependent manner.
- 15 19. A method for protecting cisplatin from degradation comprising covalently attaching said active agent to a polypeptide.
20. A method for controlling release of cisplatin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cisplatin to said polypeptide.
- 20 21. A method for delivering cisplatin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cisplatin covalently attached to said polypeptide.
22. The method of claim 21 wherein cisplatin is released from said composition
25 by an enzyme-catalyzed release.

CW097P

23. The method of claim 21 wherein cisplatin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

 A composition comprising a polypeptide and cisplatin covalently attached to the polypeptide. Also provided is a method for delivery of cisplatin to a patient comprising administering to the patient a composition comprising a polypeptide and cisplatin covalently attached to the polypeptide. Also provided is a method for protecting cisplatin
15 from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cisplatin from a composition comprising covalently attaching it to the polypeptide.

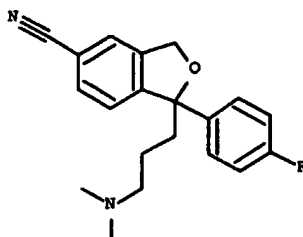
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CITALOPRAM AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to citalopram, as well as methods for protecting and administering citalopram. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Citalopram is a known pharmaceutical agent that is used in the treatment of
15 depression. Its chemical name is 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-
dihydro-5-isobenzofurancarbonitrile. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW098P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW098P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW098P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (citalopram) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching citalopram to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising citalopram microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and citalopram covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Citalopram preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW098P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting citalopram from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering citalopram to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, citalopram is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, citalopram is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and citalopram is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, citalopram is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, citalopram is released from the composition in a

CW098P

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching citalopram to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, citalopram and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW098P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize citalopram and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of citalopram. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Citalopram is the subject of GB 1526331 (1978), GB 1486 (1976), and EP 171943 B (1988), herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises citalopram covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

CW098P

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior
10 and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

CW098P

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- Ionizing amino acids can be selected for pH controlled peptide unfolding.
- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
- 15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
- 20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
- 25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

CW098P

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

CW098P

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

CW098P

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW098P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-citalopram conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW098P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW098P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW098P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW098P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 citalopram covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein citalopram is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein citalopram is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing citalopram from said composition in a pH-dependent manner.

15 19. A method for protecting citalopram from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of citalopram from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching citalopram to said polypeptide.

20 21. A method for delivering citalopram to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
citalopram covalently attached to said polypeptide.

25 22. The method of claim 21 wherein citalopram is released from said composition by an enzyme-catalyzed release.

CW098P

23. The method of claim 21 wherein citalopram is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 **Abstract**

 A composition comprising a polypeptide and citalopram covalently attached to the polypeptide. Also provided is a method for delivery of citalopram to a patient comprising administering to the patient a composition comprising a polypeptide and citalopram covalently attached to the polypeptide. Also provided is a method for
15 protecting citalopram from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of citalopram from a composition comprising covalently attaching it to the polypeptide.

CW099P

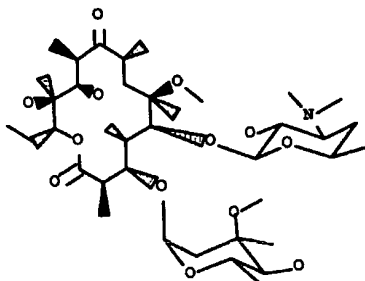
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CLARITHROMYCIN

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to clarithromycin, as well as methods for protecting and administering clarithromycin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the
10 usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Clarithromycin is a known pharmaceutical agent that is used in the treatment of
15 bacterial infection. Its chemical name is 6-O-methylerythromycin. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
20 and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW099P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW099P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW099P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (clarithromycin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching clarithromycin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising clarithromycin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and clarithromycin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Clarithromycin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW099P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting clarithromycin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering clarithromycin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, clarithromycin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, clarithromycin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and clarithromycin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, clarithromycin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, clarithromycin is

CW099P

released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug
5 conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching clarithromycin to a side chain of an amino acid to form an active
10 agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride
(NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, clarithromycin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

CW099P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize clarithromycin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of clarithromycin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Clarithromycin is the subject of EP 41355 B (1983) and EP 293885 B (1993), and US application number 58499 (1987), herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises clarithromycin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

20 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

CW099P

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

CW099P

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

- 5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- Ionizing amino acids can be selected for pH controlled peptide unfolding.
- 10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
- 15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
- 20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
- 25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

CW099P

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

CW099P

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

CW099P

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, clarithromycin is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW099P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-clarithromycin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW099P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW099P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 clarithromycin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein clarithromycin is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW099P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein clarithromycin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing clarithromycin from said composition in a pH-dependent manner.

15 19. A method for protecting clarithromycin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of clarithromycin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching clarithromycin to said polypeptide.

20 21. A method for delivering clarithromycin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
clarithromycin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein clarithromycin is released from said composition by an enzyme-catalyzed release.

CW099P

23. The method of claim 21 wherein clarithromycin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW099P

Abstract

A composition comprising a polypeptide and clarithromycin covalently attached to the polypeptide. Also provided is a method for delivery of clarithromycin to a patient comprising administering to the patient a composition comprising a polypeptide and
5 clarithromycin covalently attached to the polypeptide. Also provided is a method for protecting clarithromycin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of clarithromycin from a composition comprising covalently attaching it to the polypeptide.

CW100P

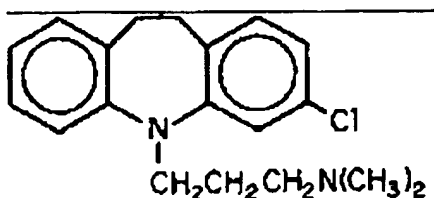
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CLOMIPRAMINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to clomipramine, as well as methods for protecting and administering clomipramine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Clomipramine is a known pharmaceutical agent that is used in the treatment of obsessive-compulsive disorder. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

CW100P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW100P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW100P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (clomipramine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching clomipramine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising clomipramine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and clomipramine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Clomipramine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW100P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting clomipramine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering clomipramine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, clomipramine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, clomipramine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and clomipramine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, clomipramine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, clomipramine is released

CW100P

from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching clomipramine to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
(NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, clomipramine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

CW100P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize clomipramine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of clomipramine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises clomipramine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW100P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
25 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

CW100P

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW100P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

CW100P

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-clomipramine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW100P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW100P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW100P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 clomipramine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein clomipramine is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW100P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.
13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.
- 5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.
15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.
- 10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.
17. The composition of claim 1 wherein clomipramine is conformationally protected by folding of said polypeptide about said active agent.
18. The composition of claim 1 wherein said polypeptide is capable of releasing clomipramine from said composition in a pH-dependent manner.
- 15 19. A method for protecting clomipramine from degradation comprising covalently attaching said active agent to a polypeptide.
20. A method for controlling release of clomipramine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching clomipramine to said polypeptide.
- 20 21. A method for delivering clomipramine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
clomipramine covalently attached to said polypeptide.
22. The method of claim 21 wherein clomipramine is released from said
25 composition by an enzyme-catalyzed release.

CW100P

23. The method of claim 21 wherein clomipramine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW100P

Abstract

A composition comprising a polypeptide and clomipramine covalently attached to the polypeptide. Also provided is a method for delivery of clomipramine to a patient comprising administering to the patient a composition comprising a polypeptide and
5 clomipramine covalently attached to the polypeptide. Also provided is a method for protecting clomipramine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of clomipramine from a composition comprising covalently attaching it to the polypeptide.

CW101P

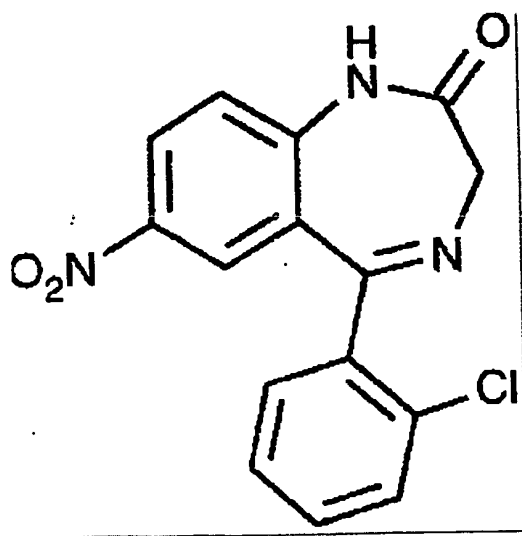
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CLONAZEPAM AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to clonazepam, as well as methods for protecting and administering clonazepam. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Clonazepam is a known pharmaceutical agent that is used in the treatment of epilepsy. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability

CW101P

of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified

CW101P

amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that

CW101P

incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (clonazepam) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching clonazepam to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising clonazepam microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and clonazepam covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a

CW101P

heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

5 Clonazepam preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is
10 an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The
15 microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be
20 conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

 The invention also provides a method for protecting clonazepam from degradation comprising covalently attaching it to a polypeptide.

25 The invention also provides a method for delivering clonazepam to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, clonazepam is released from the composition by

CW101P

an enzyme-catalyzed release. In another preferred embodiment, clonazepam is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and clonazepam is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, clonazepam is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, clonazepam is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching clonazepam to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, clonazepam and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a

CW101P

carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

5 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

10 The present invention provides several benefits for active agent delivery. First, the invention can stabilize clonazepam and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of clonazepam. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also
15 allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises clonazepam covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a
20 heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and
25 turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

CW101P

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW101P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW101P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW101P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, clonazepam is covalently attached to the polypeptide via the amino or nitro group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW101P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-clonazepam conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW101P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW101P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

CW101P

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW101P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 clonazepam covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein clonazepam is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW101P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein clonazepam is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing clonazepam from said composition in a pH-dependent manner.

15 19. A method for protecting clonazepam from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of clonazepam from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching clonazepam to said polypeptide.

20 21. A method for delivering clonazepam to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
clonazepam covalently attached to said polypeptide.

25 22. The method of claim 21 wherein clonazepam is released from said composition by an enzyme-catalyzed release.

CW101P

23. The method of claim 21 wherein clonazepam is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

A composition comprising a polypeptide and clonazepam covalently attached to the polypeptide. Also provided is a method for delivery of clonazepam to a patient comprising administering to the patient a composition comprising a polypeptide and clonazepam covalently attached to the polypeptide. Also provided is a method for
15 protecting clonazepam from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of clonazepam from a composition comprising covalently attaching it to the polypeptide.

CW102P

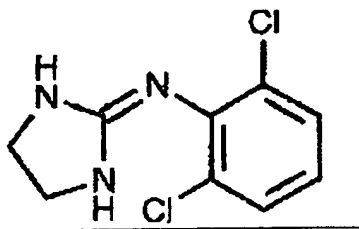
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CLONIDINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to clonidine, as well as methods for protecting and administering clonidine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

15 Clonidine is a known pharmaceutical agent that is used in the treatment of hypertension. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

CW102P

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW102P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

CW102P

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (clonidine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching clonidine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising clonidine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and clonidine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Clonidine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW102P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting clonidine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering clonidine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, clonidine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, clonidine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and clonidine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, clonidine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, clonidine is released from the composition in a sustained release.

CW102P

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

5 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching clonidine to a side chain of an amino acid to form an active agent/amino acid complex;
- 10 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a
15 second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, clonidine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular
20 transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the
25 glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

CW102P

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize clonidine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of clonidine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises clonidine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

CW102P

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

CW102P

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

25

CW102P

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

CW102P

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

CW102P

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, clonidine is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

CW102P

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
5 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known
10 intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active
15 agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the
20 catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-clonidine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

25 Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW102P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

CW102P

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 clonidine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein clonidine is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

CW102P

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein clonidine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing clonidine from said composition in a pH-dependent manner.

15 19. A method for protecting clonidine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of clonidine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching clonidine to said polypeptide.

20 21. A method for delivering clonidine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
clonidine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein clonidine is released from said composition by an enzyme-catalyzed release.

CW102P

23. The method of claim 21 wherein clonidine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW102P

Abstract

A composition comprising a polypeptide and clonidine covalently attached to the polypeptide. Also provided is a method for delivery of clonidine to a patient comprising administering to the patient a composition comprising a polypeptide and clonidine covalently attached to the polypeptide. Also provided is a method for protecting
5 clonidine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of clonidine from a composition comprising covalently attaching it to the polypeptide.

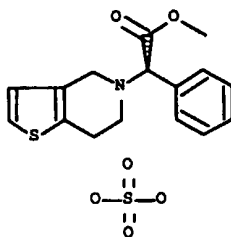
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CLOPIDOGREL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to clopidogrel, as well as methods for protecting and administering clopidogrel. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Clopidogrel is a known pharmaceutical agent that is used in the treatment of
15 thrombosis and stroke. Its chemical name is (S)- α -(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid methyl ester sulfate (1:1). Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

CW103P

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (clopidogrel) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching clopidogrel to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising clopidogrel microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and clopidogrel covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Clopidogrel preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

CW103P

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting clopidogrel from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering clopidogrel to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, clopidogrel is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, clopidogrel is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and clopidogrel is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, clopidogrel is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, clopidogrel is released from the composition in a

CW103P

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching clopidogrel to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, clopidogrel and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First,
5 the invention can stabilize clopidogrel and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of clopidogrel. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 Clopidogrel is the subject of U.S. Patent Numbers 4,529,596, 4,847,265, and 5,576,328, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises clopidogrel covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more
15 naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the
20 local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

25 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

- 5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
- 10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
- 15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

- Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
- 20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
- 25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

CW103P

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

CW103P

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

CW103P

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
25 short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-clopidogrel conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

CW103P

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 clopidogrel covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein clopidogrel is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein clopidogrel is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing clopidogrel from said composition in a pH-dependent manner.

15 19. A method for protecting clopidogrel from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of clopidogrel from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching clopidogrel to said polypeptide.

20 21. A method for delivering clopidogrel to a patient comprising administering to said patient a composition comprising:

 a polypeptide; and

 clopidogrel covalently attached to said polypeptide.

25 22. The method of claim 21 wherein clopidogrel is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein clopidogrel is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and clopidogrel covalently attached to the polypeptide. Also provided is a method for delivery of clopidogrel to a patient comprising administering to the patient a composition comprising a polypeptide and
5 clopidogrel covalently attached to the polypeptide. Also provided is a method for protecting clopidogrel from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of clopidogrel from a composition comprising covalently attaching it to the polypeptide.

CW104P

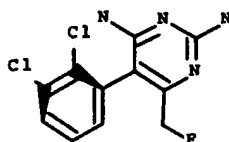
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING A SODIUM CHANNEL BLOCKER AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to a sodium channel blocker, as well as methods for protecting and administering a sodium channel blocker. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and
10 occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 The sodium channel blocker of the present invention is a known pharmaceutical
15 agent that is used in the treatment of pain. Its chemical name is (5R)-5-(2,3-dichlorophenyl)-6-(fluoromethyl)-2,4-pyrimidinediamine. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human
20 growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

CW104P

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (a sodium channel blocker) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching a sodium channel blocker to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In
10 certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier
15 peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising a sodium channel blocker microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and a sodium channel blocker covalently attached to the polypeptide. Preferably, the polypeptide is (i)
20 an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

25 A sodium channel blocker preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached

to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

5 The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

10 Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

15 The invention also provides a method for protecting a sodium channel blocker from degradation comprising covalently attaching it to a polypeptide.

 The invention also provides a method for delivering a sodium channel blocker to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently
20 attached to the polypeptide. In a preferred embodiment, a sodium channel blocker is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, a sodium channel blocker is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and a sodium channel
25 blocker is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, a sodium channel blocker is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, a sodium channel blocker is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an

CW104P

adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching a sodium channel blocker to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, a sodium channel blocker and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First,
5 the invention can stabilize a sodium channel blocker and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of a sodium channel blocker. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites
10 of action.

A sodium channel blocker is the subject of WO 97/9317 (1997), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises a sodium channel blocker covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a
15 homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have
20 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior
10 and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

CW104P

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
5 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-
10 terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the
15 preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the
20 peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid
25 moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively,
30 the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

CW104P

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, a sodium channel blocker is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating
5 the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect
10 that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own
15 associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more
20 adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance
25 absorption of the peptides.

Preferably, the resultant peptide-a sodium channel blocker conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW104P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to
5 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in
15 ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with
20 triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of
25 other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW104P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

5 There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 a sodium channel blocker covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
15 one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein a sodium channel blocker is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
10. The composition of claim 9 wherein said microencapsulating agent is
20 selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein a sodium channel blocker is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing a sodium channel blocker from said composition in a pH-dependent manner.

15 19. A method for protecting a sodium channel blocker from degradation comprising covalently attaching said active agent to a polypeptide.

20 20. A method for controlling release of a sodium channel blocker from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching a sodium channel blocker to said polypeptide.

21. A method for delivering a sodium channel blocker to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
a sodium channel blocker covalently attached to said polypeptide.

25 22. The method of claim 21 wherein a sodium channel blocker is released from said composition by an enzyme-catalyzed release.

CW104P

23. The method of claim 21 wherein a sodium channel blocker is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 **Abstract**

15 A composition comprising a polypeptide and a sodium channel blocker covalently attached to the polypeptide. Also provided is a method for delivery of a sodium channel blocker to a patient comprising administering to the patient a composition comprising a polypeptide and a sodium channel blocker covalently attached to the polypeptide. Also provided is a method for protecting a sodium channel blocker from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of a sodium channel blocker from a composition comprising covalently attaching it to the polypeptide.

CW001P

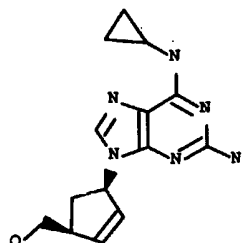
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
ABACAVIR SULFATE AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to abacavir sulfate, as well as methods for protecting and administering abacavir sulfate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Abacavir sulfate is a known pharmaceutical agent – a carbocyclic 2'-
15 deoxyguanosine nucleoside analogue that is a reverse transcriptase inhibitor used in the treatment of HIV. Its chemical name is (1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol. Its structure is as follows:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

10 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
15 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
20 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
25 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle
30 size of the active agent delivery system. Variable molecular weights have unpredictable

CW001P

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent (abacavir sulfate) to a polymer of peptides or amino acids. The invention is distinguished from the above mentioned technologies by virtue of covalently attaching abacavir sulfate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
- 15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising abacavir microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and abacavir sulfate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids,
- 25 (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

CW001P

abacavir sulfate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting abacavir sulfate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering abacavir sulfate to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, abacavir sulfate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, abacavir sulfate is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and abacavir sulfate is released from the composition by dissolution of the microencapsulating agent. In another preferred

embodiment, abacavir sulfate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, abacavir sulfate is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching abacavir sulfate to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, abacavir sulfate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

CW001P

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize abacavir sulfate and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of abacavir sulfate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Abacavir sulfate is the subject of U.S. Patent Numbers 5,034,394 and 5,089,500, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises abacavir sulfate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW001P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, abacavir sulfate is covalently attached to the polypeptide via its alcohol group or, alternatively, its amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

CW001P

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-abacavir sulfate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW001P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to
5 0°C. The solution can then be treated with diisopropylcarbodiimide and
hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be
stirred for several hours at room temperature, the urea by-product filtered off, the product
precipitated out in ether and purified using gel permeation chromatography (GPC) or
dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
followed by the amine bioactive agent. The reaction can then be stirred for several hours
at room temperature, the urea by-product filtered off, the product precipitated out in ether
15 and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene
produces a chloroformate, which when reacted with the N-terminus of the peptide
produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with
20 triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
added slowly and the solution stirred at room temperature for several hours. The product
is then precipitated out in ether. The crude product is suitably deprotected and purified
using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of
25 other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
solvents such as chloroform. Examples of other activating agents include
dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

CW001P

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 abacavir sulfate covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein abacavir sulfate is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein abacavir sulfate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing abacavir sulfate from said composition in a pH-dependent manner.

15 19. A method for protecting abacavir sulfate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of abacavir sulfate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching abacavir sulfate to said polypeptide.

20 21. A method for delivering abacavir sulfate to a patient comprising administering to said patient a composition comprising:
 a polypeptide; and
 abacavir sulfate covalently attached to said polypeptide.

25 22. The method of claim 21 wherein abacavir sulfate is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein abacavir sulfate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

A composition comprising a polypeptide and abacavir sulfate covalently attached to the polypeptide. Also provided is a method for delivery of abacavir sulfate to a patient comprising administering to the patient a composition comprising a polypeptide and abacavir sulfate covalently attached to the polypeptide. Also provided is a method for
15 protecting abacavir sulfate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of abacavir sulfate from a composition comprising covalently attaching it to a polypeptide.

CW002P

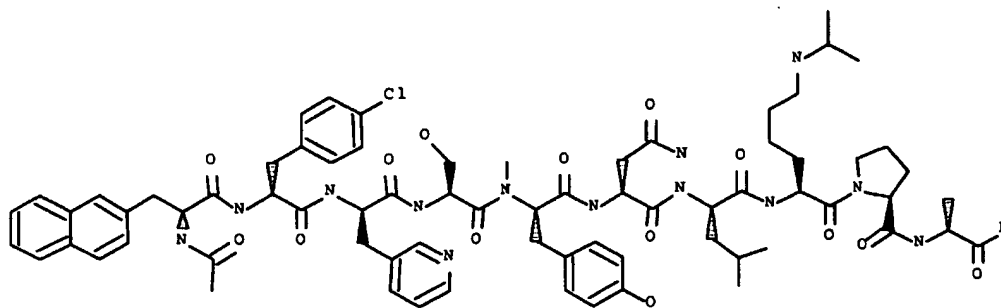
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING ABARELIX AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to abarelix, as well as methods for protecting and administering abarelix. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Abarelix is a known pharmaceutical agent that is used in the treatment of prostate cancer, acting as a gonadotropin-releasing hormone antagonist. Its chemical name is N-acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-N-methyl-L-tyrosyl-D-asparagynyl-L-N6-(1-methylethyl)-L-lysyl-L-prolyl-D-alaninamide. Abarelix is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is as follows:



CW002P

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release

CW002P

through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide

CW002P

linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

- It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.
- Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent (abarelix) to a polymer of peptides or amino acids. The invention is distinguished from the above mentioned technologies by virtue of covalently attaching abarelix to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection.
- In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- Alternatively, the present invention provides a pharmaceutical composition comprising abarelix microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and abarelix covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,

(ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Abarelix preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting abarelix from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering abarelix to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the

CW002P

polypeptide. In a preferred embodiment, abarelix is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, abarelix is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a

5 microencapsulating agent and abarelix is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, abarelix is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, abarelix is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant

10 covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method

15 comprises the steps of:

- (a) attaching abarelix to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- 20 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, abarelix and a second active agent can be copolymerized in step (c). In another

25 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,

30 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a

carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

5 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

10 The present invention provides several benefits for active agent delivery. First, the invention can stabilize abarelix and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of abarelix. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted
15 delivery of active agents to specific sites of action.

The composition of the invention comprises abarelix covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a
20 heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and
25 turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

CW002P

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

CW002P

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

CW002P

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

CW002P

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, abarelix is covalently attached to the polypeptide via the free alcohol group or, alternatively, through one of its amino groups.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-abarelix conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

CW002P

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to
5 0°C. The solution can then be treated with diisopropylcarbodiimide and
hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be
stirred for several hours at room temperature, the urea by-product filtered off, the product
precipitated out in ether and purified using gel permeation chromatography (GPC) or
dialysis.

10 Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
followed by the amine bioactive agent. The reaction can then be stirred for several hours
at room temperature, the urea by-product filtered off, the product precipitated out in ether
15 and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene
produces a chloroformate, which when reacted with the N-terminus of the peptide
produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with
20 triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then
added slowly and the solution stirred at room temperature for several hours. The product
is then precipitated out in ether. The crude product is suitably deprotected and purified
using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of
25 other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated
solvents such as chloroform. Examples of other activating agents include
dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which
5 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

10 The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using GPC or dialysis.

15 Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

20 Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

25 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 abarelix covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein abarelix is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein abarelix is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing abarelix from said composition in a pH-dependent manner.

15 19. A method for protecting abarelix from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of abarelix from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching abarelix to said polypeptide.

20 21. A method for delivering abarelix to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
abarelix covalently attached to said polypeptide.

25 22. The method of claim 21 wherein abarelix is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein abarelix is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10

Abstract

A composition comprising a polypeptide and abarelix covalently attached to the polypeptide. Also provided is a method for delivery of abarelix to a patient comprising administering to the patient a composition comprising a polypeptide and abarelix
15 covalently attached to the polypeptide. Also provided is a method for protecting abarelix from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of abarelix from a composition comprising covalently attaching it to the polypeptide.

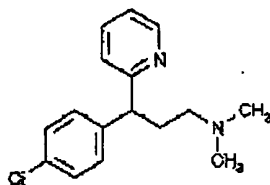
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
CHLORPHENIRAMINE AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to chlorpheniramine, as well as methods for protecting and administering chlorpheniramine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Chlorpheniramine is a known pharmaceutical agent that is used in the treatment of
15 nasal congestion. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (chlorpheniramine tannate) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching chlorpheniramine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising chlorpheniramine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and chlorpheniramine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Chlorpheniramine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active

agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet
5 another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino
10 acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In
15 another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting chlorpheniramine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering chlorpheniramine to a
20 patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, chlorpheniramine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, chlorpheniramine is released in a time-dependent manner based on the pharmacokinetics
25 of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and chlorpheniramine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, chlorpheniramine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, chlorpheniramine is

released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug
5 conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching chlorpheniramine to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
(NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, chlorpheniramine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize chlorpheniramine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of chlorpheniramine tannate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises chlorpheniramine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the

protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of
15 maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the
20 amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.
25 Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a
25 short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-chlorpheniramine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 chlorpheniramine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein chlorpheniramine is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein chlorpheniramine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing chlorpheniramine from said composition in a pH-dependent manner.

15 19. A method for protecting chlorpheniramine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of chlorpheniramine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching chlorpheniramine to said polypeptide.

20 21. A method for delivering chlorpheniramine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
chlorpheniramine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein chlorpheniramine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein chlorpheniramine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and chlorpheniramine covalently attached to the polypeptide. Also provided is a method for delivery of chlorpheniramine to a patient comprising administering to the patient a composition comprising a

5 polypeptide and chlorpheniramine covalently attached to the polypeptide. Also provided is a method for protecting chlorpheniramine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of chlorpheniramine from a composition comprising covalently attaching it to the polypeptide.

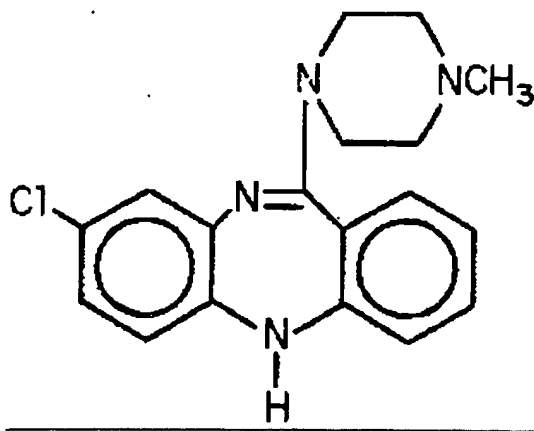
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CLOZAPINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to clozapine, as well as methods for protecting and administering clozapine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Clozapine is a known pharmaceutical agent that is used in the treatment of
15 psychotic disorders. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;

and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

10 SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (clozapine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching clozapine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising clozapine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and clozapine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Clozapine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a
5 carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-
10 terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
15 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
20 composition in a pH-dependent manner.

The invention also provides a method for protecting clozapine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering clozapine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a
25 composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, clozapine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, clozapine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release.

In another preferred embodiment, the composition further comprises a microencapsulating agent and clozapine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, clozapine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, clozapine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

10 The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching clozapine to a side chain of an amino acid to form an active agent/amino acid complex;
- 15 (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, clozapine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize clozapine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of clozapine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises clozapine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, clozapine is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-clozapine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 clozapine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein clozapine is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein clozapine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing clozapine from said composition in a pH-dependent manner.

15 19. A method for protecting clozapine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of clozapine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching clozapine to said polypeptide.

20 21. A method for delivering clozapine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
clozapine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein clozapine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein clozapine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and clozapine covalently attached to the polypeptide. Also provided is a method for delivery of clozapine to a patient comprising administering to the patient a composition comprising a polypeptide and clozapine covalently attached to the polypeptide. Also provided is a method for protecting
5 clozapine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of clozapine from a composition comprising covalently attaching it to the polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING COLESTIPOL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to colestipol, as well as methods for protecting and administering colestipol. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Colestipol is a known pharmaceutical agent that is used in the treatment of
15 hypercholesterolemia. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Colestipol hydrochloride is a copolymer of diethylenetriamine and 1-chloro-2,3-epoxypropane that contains secondary and tertiary amines with approximately 1 out of 5 amine nitrogens protonated with chloride.

20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical
25 compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken

under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even
5 reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of
10 cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

15 Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release
20 through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several
25 shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent

in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

- 5 In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral
- 10 administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.
- 15 This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the
- 20 active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

- It is also important to control the molecular weight, molecular size and particle
- 25 size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.
- 30 Particle size not only becomes a problem with injectable drugs, as in the HAR

application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
5 (colestipol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching colestipol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
10 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

15 Alternatively, the present invention provides a pharmaceutical composition comprising colestipol microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and colestipol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
20 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Colestipol preferably is covalently attached to a side chain, the N-terminus or the
25 C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is

an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a
5 microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet,
10 an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting colestipol from degradation
15 comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering colestipol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, colestipol is released from the composition by
20 an enzyme-catalyzed release. In another preferred embodiment, colestipol is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and colestipol is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, colestipol is released
25 from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, colestipol is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method

5 comprises the steps of:

(a) attaching colestipol to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, colestipol and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize colestipol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of colestipol.

- 5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

- The composition of the invention comprises colestipol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
10 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have
15 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
20 constitute the tertiary structure.

- Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino
25 acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will
5 ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of
10 the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a
15 synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level
20 of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
25 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
 5 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

10

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
 15 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could
 20 conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, colestipol is covalently attached to the polypeptide via one of its amino groups.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
5 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
10 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
15 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

20 Preferably, the resultant peptide-colestipol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

25 An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

- 10 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
15 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

- Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include
20 dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

- There have been over 30 different γ -alkyl glutamates prepared any one of which
25 may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.

- 5 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

- 10 γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

- 15 γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

- 20 Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 colestipol covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein colestipol is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein colestipol is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing colestipol from said composition in a pH-dependent manner.

15 19. A method for protecting colestipol from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of colestipol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching colestipol to said polypeptide.

20 21. A method for delivering colestipol to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
colestipol covalently attached to said polypeptide.

25 22. The method of claim 21 wherein colestipol is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein colestipol is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and colestipol covalently attached to the polypeptide. Also provided is a method for delivery of colestipol to a patient comprising administering to the patient a composition comprising a polypeptide and colestipol covalently attached to the polypeptide. Also provided is a method for protecting
5 colestipol from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of colestipol from a composition comprising covalently attaching it to the polypeptide.

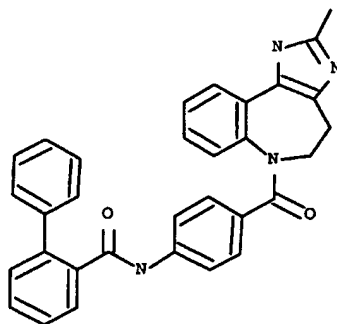
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CONIVAPTAN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to conivaptan, as well as methods for protecting and administering conivaptan. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Conivaptan is a known pharmaceutical agent that is used in the treatment of
15 congestive heart failure and hyponatremia. Its chemical name is N-[1,1'-biphenyl]-2-yl-4-[(4,5-dihydro-2-methylimidazo[4,5-d][1]benzazepin-6(1H)-yl)carbonyl]-benzamide. Its structure is:



20 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group.

This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent
10 (conivaptan) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching conivaptan to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
15 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising conivaptan microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and conivaptan covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
25 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Conivaptan preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting conivaptan from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering conivaptan to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, conivaptan is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, conivaptan is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and conivaptan is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, conivaptan is

released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, conivaptan is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching conivaptan to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, conivaptan and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize conivaptan and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of conivaptan. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Conivaptan is the subject of EP 709386 A (1996), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises conivaptan covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is
5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

5 The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex,
10 PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
15 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
20 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
25 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-conivaptan conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

5 **Acid/N-terminus conjugation**

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product
10 precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
15 followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene
20 produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified
25 using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated

solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

5 Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered,
10 dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for
15 several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes
20 homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically
25 overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 conivaptan covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein conivaptan is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein conivaptan is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing conivaptan from said composition in a pH-dependent manner.

15 19. A method for protecting conivaptan from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of conivaptan from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching conivaptan to said polypeptide.

20 21. A method for delivering conivaptan to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
conivaptan covalently attached to said polypeptide.

25 22. The method of claim 21 wherein conivaptan is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein conivaptan is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

 A composition comprising a polypeptide and conivaptan covalently attached to the polypeptide. Also provided is a method for delivery of conivaptan to a patient comprising administering to the patient a composition comprising a polypeptide and conivaptan covalently attached to the polypeptide. Also provided is a method for
15 protecting conivaptan from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of conivaptan from a composition comprising covalently attaching it to the polypeptide.

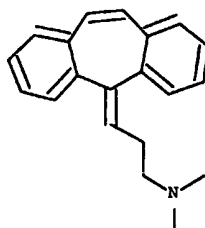
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
CYCLOBENZAPRINE AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cyclobenzaprine, as well as methods for protecting and administering cyclobenzaprine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Cyclobenzaprine is a known pharmaceutical agent that is used in the treatment of
15 muscle spasm. Its chemical name is 3-(5H-dibenzo[a,d]cyclohepten-5-ylidene)-N,N-dimethyl-1-propanamine. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cyclobenzaprine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cyclobenzaprine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cyclobenzaprine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cyclobenzaprine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cyclobenzaprine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cyclobenzaprine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cyclobenzaprine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cyclobenzaprine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cyclobenzaprine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cyclobenzaprine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cyclobenzaprine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cyclobenzaprine is

released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug
5 conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching cyclobenzaprime to a side chain of an amino acid to form an active
10 agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cyclobenzaprime and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize cyclobenzaprine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cyclobenzaprine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises cyclobenzaprine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.
- 25

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cyclobenzaprine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cyclobenzaprine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cyclobenzaprine is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cyclobenzaprine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cyclobenzaprine from said composition in a pH-dependent manner.

15 19. A method for protecting cyclobenzaprine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cyclobenzaprine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cyclobenzaprine to said polypeptide.

20 21. A method for delivering cyclobenzaprine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cyclobenzaprine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cyclobenzaprine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cyclobenzaprine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cyclobenzaprine covalently attached to the polypeptide. Also provided is a method for delivery of cyclobenzaprine to a patient comprising administering to the patient a composition comprising a polypeptide and cyclobenzaprine covalently attached to the polypeptide. Also provided is a method for protecting cyclobenzaprine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cyclobenzaprine from a composition comprising covalently attaching it to the polypeptide.

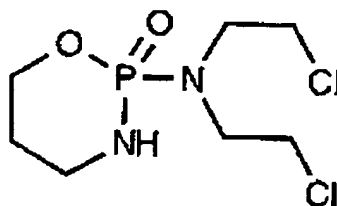
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CYCLOPHOSPHAMIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cyclophosphamide, as well as methods for protecting and administering cyclophosphamide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Cyclophosphamide is a known pharmaceutical agent that is used in the treatment
15 of myeloproliferative and lymphoproliferative disorders and solid malignancies. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cyclophosphamide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cyclophosphamide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cyclophosphamide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cyclophosphamide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cyclophosphamide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active

agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet
5 another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino
10 acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In
15 another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cyclophosphamide from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cyclophosphamide to a
20 patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cyclophosphamide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cyclophosphamide is released in a time-dependent manner based on the pharmacokinetics
25 of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cyclophosphamide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cyclophosphamide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cyclophosphamide is

released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug
5 conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching cyclophosphamide to a side chain of an amino acid to form an active
10 agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride
(NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cyclophosphamide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cyclophosphamide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cyclophosphamide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises cyclophosphamide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of
15 one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.
- 25

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, cyclophosphamide is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
5 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the
10 transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active
15 agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the
20 catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cyclophosphamide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

25 Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cyclophosphamide covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cyclophosphamide is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cyclophosphamide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cyclophosphamide from said composition in a pH-dependent manner.

15 19. A method for protecting cyclophosphamide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cyclophosphamide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cyclophosphamide to said polypeptide.

20 21. A method for delivering cyclophosphamide to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cyclophosphamide covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cyclophosphamide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cyclophosphamide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cyclophosphamide covalently attached to the polypeptide. Also provided is a method for delivery of cyclophosphamide to a patient comprising administering to the patient a composition comprising a

5 polypeptide and cyclophosphamide covalently attached to the polypeptide. Also provided is a method for protecting cyclophosphamide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cyclophosphamide from a composition comprising covalently attaching it to the polypeptide.

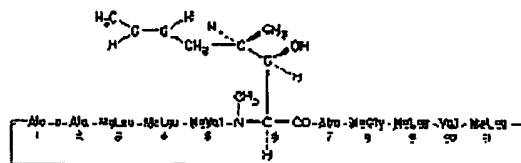
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING CYCLOSPORINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cyclosporine, as well as methods for protecting and administering cyclosporine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Cyclosporine is a known pharmaceutical agent that is used in the treatment of
15 prevention of rejection of kidney, liver or heart allografts. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase
5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly
10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the
15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and
20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can
25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some
5 technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically
10 require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the
15 gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The
20 released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that
25 incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly
30 or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (cyclosporine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cyclosporine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cyclosporine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and cyclosporine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Cyclosporine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting cyclosporine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cyclosporine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cyclosporine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cyclosporine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and cyclosporine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cyclosporine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cyclosporine is released from the

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cyclosporine to a side chain of an amino acid to form an active
10 agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cyclosporine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and
20 wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a
25 carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.
30 The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cyclosporine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cyclosporine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises cyclosporine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

- 5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

- 10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

- 15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

- 20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.
- 25

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, cyclosporine is covalently attached to the polypeptide via the hydroxyl group or, alternatively, via an artificial linker.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cyclosporine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cyclosporine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cyclosporine is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cyclosporine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cyclosporine from said composition in a pH-dependent manner.

15 19. A method for protecting cyclosporine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cyclosporine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cyclosporine to said polypeptide.

20 21. A method for delivering cyclosporine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cyclosporine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cyclosporine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cyclosporine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and cyclosporine covalently attached to the polypeptide. Also provided is a method for delivery of cyclosporine to a patient comprising administering to the patient a composition comprising a polypeptide and cyclosporine covalently attached to the polypeptide. Also provided is a method for
5 protecting cyclosporine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cyclosporine from a composition comprising covalently attaching it to the polypeptide.

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING DALTEPARIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to dalteparin, as well as methods for protecting and administering dalteparin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Dalteparin (also known as heparin) is a known pharmaceutical agent that is used
15 in the treatment of prevention of ischemic complications, due to blood clot formation in patients with unstable angina and non-Q-wave myocardial infarction receiving concurrent aspirin therapy. It is a natural product that is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art.

 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical
25 agent, an adjuvant, or an inhibitor.

 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

5 Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres,
10 liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

 Active agent delivery systems also provide the ability to control the release of the
15 active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified
20 amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

 Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the
25 microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some

technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR

application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
5 (dalteparin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching dalteparin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
10 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

15 Alternatively, the present invention provides a pharmaceutical composition comprising dalteparin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and dalteparin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
20 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Dalteparin preferably is covalently attached to a side chain, the N-terminus or the
25 C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is

an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a
5 microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet,
10 an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting dalteparin from degradation
15 comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering dalteparin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, dalteparin is released from the composition by
20 an enzyme-catalyzed release. In another preferred embodiment, dalteparin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and dalteparin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, dalteparin is released
25 from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, dalteparin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method
5 comprises the steps of:

(a) attaching dalteparin to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, dalteparin and a second active agent can be copolymerized in step (c). In another
15 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,
20 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize dalteparin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of dalteparin.

- 5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Dalteparin is the subject of EP 14184 B (1989), herein incorporated by reference, which describes how to make that drug.

- 10 The composition of the invention comprises dalteparin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or
15 more naturally occurring amino acids and one or more synthetic amino acids.

- Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

- Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

25

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group
5 selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples,
10 the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate
15 with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier.
20 Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will
25 then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is
30 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action
5 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is
10 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or
15 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

20 In the present invention, dalteparin is covalently attached to the polypeptide via any free hydroxyl, amino, or carboxyl group or, alternatively, via an artificial linker.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer
25 can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-dalteparin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone; filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 dalteparin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein dalteparin is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein dalteparin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing dalteparin from said composition in a pH-dependent manner.

15 19. A method for protecting dalteparin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of dalteparin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching dalteparin to said polypeptide.

20 21. A method for delivering dalteparin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
dalteparin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein dalteparin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein dalteparin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and dalteparin covalently attached to the polypeptide. Also provided is a method for delivery of dalteparin to a patient comprising administering to the patient a composition comprising a polypeptide and dalteparin covalently attached to the polypeptide. Also provided is a method for protecting dalteparin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of dalteparin from a composition comprising covalently attaching it to the polypeptide.

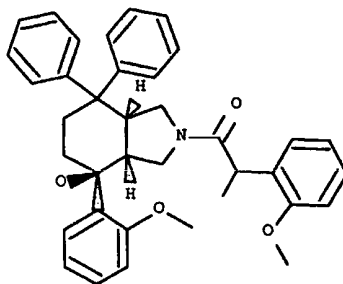
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING DAPITANT AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to dapitant, as well as methods for protecting and administering dapitant. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Dapitant is a known pharmaceutical agent that is used in the treatment of asthma. Its chemical name is [3aS-[2(R*),3aalpha,4beta,7aalpha]]-octahydro-4-(2-methoxyphenyl)-2-[2-(2-methoxyphenyl)-1-oxopropyl]-7,7-diphenyl-1H-isoindol-4-ol. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent (dapitant) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching dapitant to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection.
- 15 In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising dapitant microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and dapitant covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
- 25

Dapitant preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting dapitant from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering dapitant to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, dapitant is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, dapitant is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and dapitant is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, dapitant is released

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, dapitant is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching dapitant to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, dapitant and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize dapitant and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of dapitant. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted
10 delivery of active agents to specific sites of action.

Dapitant is the subject of WO 93/21155 (1993), herein incorporated by reference, which describes how to make that drug.

 The composition of the invention comprises dapitant covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one
15 of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

 Proteins, oligopeptides and polypeptides are polymers of amino acids that have
20 primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains
25 constitute the tertiary structure.

 Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best
5 model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior
10 and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are “pushed out” of the packed, hydrophobic protein core. All of these forces combine and
15 contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a
20 peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and
25 at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

5 Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding.

10 Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all
15 be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
20 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
25 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a
 5 macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several
 10 aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of
 15 lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's
 20 molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, dapitant is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-dapitant conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

 γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 dapitant covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein dapitant is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein dapitant is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing dapitant from said composition in a pH-dependent manner.

15 19. A method for protecting dapitant from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of dapitant from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching dapitant to said polypeptide.

20 21. A method for delivering dapitant to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
dapitant covalently attached to said polypeptide.

25 22. The method of claim 21 wherein dapitant is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein dapitant is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and dapitant covalently attached to the polypeptide. Also provided is a method for delivery of dapitant to a patient comprising administering to the patient a composition comprising a polypeptide and dapitant
5 covalently attached to the polypeptide. Also provided is a method for protecting dapitant from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of dapitant from a composition comprising covalently attaching it to the polypeptide.

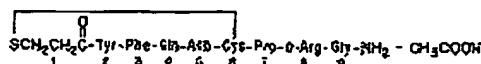
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING DESMOPRESSIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to desmopressin, as well as methods for protecting and administering desmopressin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Desmopressin is a known pharmaceutical agent that is used in the treatment of
15 urinary incontinence. Its chemical name is 1-(3-mercaptopropanic acid)-8-D-arginine-vasopressin. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

5 Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres,
10 liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

 Active agent delivery systems also provide the ability to control the release of the
15 active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified
20 amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

 Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the
25 microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some

technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR

application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent
5 (desmopressin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching desmopressin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through
10 conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

15 Alternatively, the present invention provides a pharmaceutical composition comprising desmopressin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and desmopressin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a
20 heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Desmopressin preferably is covalently attached to a side chain, the N-terminus or
25 the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is

an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a
5 microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet,
10 an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting desmopressin from
15 degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering desmopressin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, desmopressin is released from the composition
20 by an enzyme-catalyzed release. In another preferred embodiment, desmopressin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and desmopressin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment,
25 desmopressin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, desmopressin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The

adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method

5 comprises the steps of:

(a) attaching desmopressin to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

10 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, desmopressin and a second active agent can be copolymerized in step (c). In

15 another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side
20 chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

25 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize desmopressin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of desmopressin.

5 Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Desmopressin is the subject of U.S. Patent Numbers 5,047,398, 5,500,413, 5,674,850, and 5,763,407, herein incorporated by reference, which describes how to

10 make that drug.

The composition of the invention comprises desmopressin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a

15 heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and

20 turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on

25 the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the

protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant
10 force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of
15 maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the
20 amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.
25 Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, desmopressin is covalently attached to the polypeptide via an amide linkage with the amino group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-desmopressin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 desmopressin covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein desmopressin is covalently attached to a
side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein desmopressin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing desmopressin from said composition in a pH-dependent manner.

15 19. A method for protecting desmopressin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of desmopressin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching desmopressin to said polypeptide.

20 21. A method for delivering desmopressin to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
desmopressin covalently attached to said polypeptide.

25 22. The method of claim 21 wherein desmopressin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein desmopressin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and desmopressin covalently attached to the polypeptide. Also provided is a method for delivery of desmopressin to a patient comprising administering to the patient a composition comprising a polypeptide and
5 desmopressin covalently attached to the polypeptide. Also provided is a method for protecting desmopressin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of desmopressin from a composition comprising covalently attaching it to the polypeptide.

**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING DESOGESTREL
AND ETHINYL ESTRADIOL AND METHODS OF
MAKING AND USING SAME**

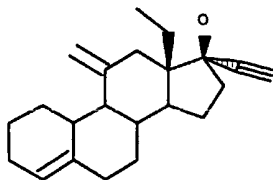
5 FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to desogestrel and ethinyl estradiol, as well as methods for protecting and administering desogestrel and ethinyl estradiol. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

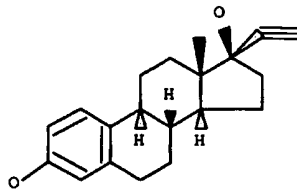
BACKGROUND OF THE INVENTION

15 Desogestrel and ethinyl estradiol are known pharmaceutical agents used together as a contraceptive.

Desogestrel's chemical name is (17 α)-13-ethyl-11-methylene-18,19-dinorpregn-4-en-20-yn-17-ol. Its structure is:



20 Ethinyl estradiol's chemical name is (17 α)-19-norpregna-1,2,5(10)-trien-20-yne-3,17-diol. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release

through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide

linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (desogestrel and ethinyl estradiol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching desogestrel and ethinyl estradiol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising desogestrel and ethinyl estradiol microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and desogestrel and ethinyl estradiol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a
5 homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Desogestrel and ethinyl estradiol preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the
10 active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol
15 and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
20 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
25 composition in a pH-dependent manner.

The invention also provides a method for protecting desogestrel and ethinyl estradiol from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering desogestrel and ethinyl estradiol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, desogestrel and ethinyl estradiol are released from the composition by an enzyme-catalyzed release. In another preferred embodiment, desogestrel and ethinyl estradiol are released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and desogestrel and ethinyl estradiol are released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, desogestrel and ethinyl estradiol are released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, desogestrel and ethinyl estradiol are released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching desogestrel and ethinyl estradiol to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, desogestrel and ethinyl estradiol and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the

polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is
5 attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

10 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

15 DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize desogestrel and ethinyl estradiol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of desogestrel and ethinyl estradiol. Furthermore, active agents can be combined to
20 produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Desogestrel are the subject of GB 1455270 (1976), herein incorporated by reference, which describes how to make that drug.

25 The composition of the invention comprises desogestrel and ethinyl estradiol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a

heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of

maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.

5 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
10 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
15 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
20 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

25 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of

the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple
5 active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain
10 length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of
15 active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate
20 weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the
25 jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to

poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

5 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
10 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
15 carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
20 specific properties to the drug delivery system.

 The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
25 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
30 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
5 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, desogestrel and ethinyl estradiol are covalently attached to the polypeptide via their hydroxyl groups.

The polypeptide carrier can be prepared using conventional techniques. A
10 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG)
15 and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal
20 epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the
25 transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the

mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-desogestrel and ethinyl estradiol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 desogestrel and ethinyl estradiol covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein desogestrel and ethinyl estradiol are covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein desogestrel and ethinyl estradiol are conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing desogestrel and ethinyl estradiol from said composition in a pH-dependent manner.

15 19. A method for protecting desogestrel and ethinyl estradiol from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of desogestrel and ethinyl estradiol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching desogestrel and ethinyl estradiol to said polypeptide.

20 21. A method for delivering desogestrel and ethinyl estradiol to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
desogestrel and ethinyl estradiol covalently attached to said polypeptide.

25 22. The method of claim 21 wherein desogestrel and ethinyl estradiol are released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein desogestrel and ethinyl estradiol are released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

 A composition comprising a polypeptide and desogestrel and ethinyl estradiol covalently attached to the polypeptide. Also provided is a method for delivery of desogestrel and ethinyl estradiol to a patient comprising administering to the patient a composition comprising a polypeptide and desogestrel and ethinyl estradiol covalently
15 attached to the polypeptide. Also provided is a method for protecting desogestrel and ethinyl estradiol from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of desogestrel and ethinyl estradiol from a composition comprising covalently attaching it to the polypeptide.

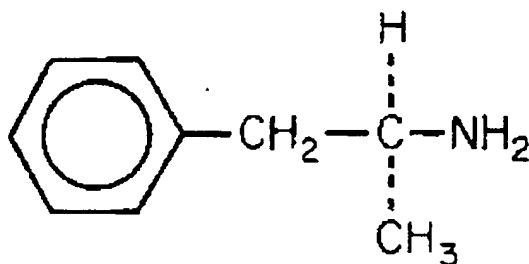
**A NOVEL PHARMACEUTICAL COMPOUND CONTAINING
DEXTROAMPHETAMINE AND METHODS OF MAKING AND USING SAME**

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to dextroamphetamine, as well as methods for protecting and administering dextroamphetamine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Dextroamphetamine is a known pharmaceutical agent that is used in the treatment
15 of narcolepsy and attention deficit hyperactivity disorder. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

- 5 Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

- The present invention provides covalent attachment of the active agent
10 (dextroamphetamine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching dextroamphetamine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the
15 stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

- 20 Alternatively, the present invention provides a pharmaceutical composition comprising dextroamphetamine microencapsulated by a polypeptide.

- The invention provides a composition comprising a polypeptide and dextroamphetamine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino
25 acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Dextroamphetamine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached
5 to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a
10 microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet,
15 an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting dextroamphetamine from
20 degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering dextroamphetamine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, dextroamphetamine is released
25 from the composition by an enzyme-catalyzed release. In another preferred embodiment, dextroamphetamine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and dextroamphetamine is released from the composition by dissolution of the microencapsulating agent. In another

preferred embodiment, dextroamphetamine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, dextroamphetamine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching dextroamphetamine to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, dextroamphetamine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize dextroamphetamine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of dextroamphetamine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises dextroamphetamine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

5 The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with
10 protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal
15 packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

 Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released.
20 The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is
25 often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

 Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery.

- 5 Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border
- 10 membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

15

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

- Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For
- 20 example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

5 The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an
10 amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example
15 above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

20 The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal
25 enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-
30 carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart
5 specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these
10 polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the
15 alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-
20 dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, dextroamphetamine is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A
25 preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-dextroamphetamine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 dextroamphetamine covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein dextroamphetamine is covalently attached
to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein dextroamphetamine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing dextroamphetamine from said composition in a pH-dependent manner.

15 19. A method for protecting dextroamphetamine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of dextroamphetamine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching dextroamphetamine to said polypeptide.

20 21. A method for delivering dextroamphetamine to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
dextroamphetamine covalently attached to said polypeptide.

25 22. The method of claim 21 wherein dextroamphetamine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein dextroamphetamine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and dextroamphetamine covalently attached to the polypeptide. Also provided is a method for delivery of dextroamphetamine to a patient comprising administering to the patient a composition

5 comprising a polypeptide and dextroamphetamine covalently attached to the polypeptide. Also provided is a method for protecting dextroamphetamine from degradation comprising covalently attaching it to a polypeptide. . Also provided is a method for controlling release of dextroamphetamine from a composition comprising covalently attaching it to the polypeptide.

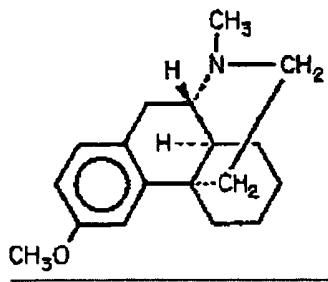
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING DEXTROMETHORPHAN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to dextromethorphan, as well as methods for protecting and administering dextromethorphan. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a
10 known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Dextromethorphan is a known pharmaceutical agent that is used in the treatment
15 of coughs. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered
product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type;
and provision for an oral dosage form when none exists. The novel pharmaceutical
compound may contain one or more of the following: another active pharmaceutical
agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase

5 markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly

10 acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the

15 active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and

20 aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can

25 also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (dextromethorphan) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching dextromethorphan to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising dextromethorphan microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and dextromethorphan covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Dextromethorphan preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active

agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet
5 another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino
10 acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In
15 another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting dextromethorphan from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering dextromethorphan to a
20 patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, dextromethorphan is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, dextromethorphan is released in a time-dependent manner based on the pharmacokinetics
25 of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and dextromethorphan is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, dextromethorphan is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, dextromethorphan is

released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching dextromethorphan to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, dextromethorphan and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize dextromethorphan and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of dextromethorphan. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

10 The composition of the invention comprises dextromethorphan covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of
15 one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the
20 conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding
25 are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational
5 entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular
10 bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has
15 maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is
20 a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious
25 chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

5 Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

10 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

15 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

20 As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

25

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-dextromethorphan conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for
5 several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
10 followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and
15 the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of
20 a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
25 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 dextromethorphan covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein dextromethorphan is covalently attached
to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein dextromethorphan is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing dextromethorphan from said composition in a pH-dependent manner.

15 19. A method for protecting dextromethorphan from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of dextromethorphan from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching dextromethorphan to said polypeptide.

20 21. A method for delivering dextromethorphan to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
dextromethorphan covalently attached to said polypeptide.

25 22. The method of claim 21 wherein dextromethorphan is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein dextromethorphan is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

- 5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and dextromethorphan covalently attached to the polypeptide. Also provided is a method for delivery of dextromethorphan to a patient comprising administering to the patient a composition comprising a
5 polypeptide and dextromethorphan covalently attached to the polypeptide. Also provided is a method for protecting dextromethorphan from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of dextromethorphan from a composition comprising covalently attaching it to the polypeptide.

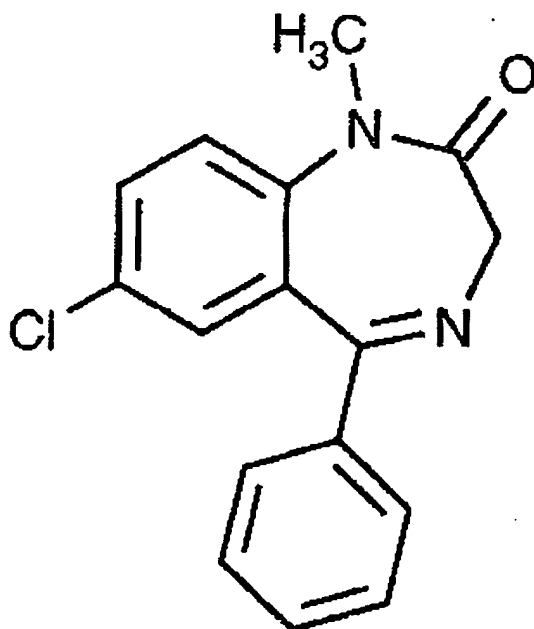
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING DIAZEPAM AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to diazepam, as well as methods for protecting and administering diazepam. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Diazepam is a known pharmaceutical agent that is used in the treatment of
15 anxiety. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release

through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide

linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (diazepam) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching diazepam to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising diazepam microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and diazepam covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide,

(ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Diazepam preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting diazepam from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering diazepam to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the

polypeptide. In a preferred embodiment, diazepam is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, diazepam is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a

5 microencapsulating agent and diazepam is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, diazepam is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, diazepam is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant

10 covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method

15 comprises the steps of:

- (a) attaching diazepam to a side chain of an amino acid to form an active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and
- 20 (c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, diazepam and a second active agent can be copolymerized in step (c). In another

25 preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine,

30 cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a

carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

5 It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

10 The present invention provides several benefits for active agent delivery. First, the invention can stabilize diazepam and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of diazepam. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also
15 allows targeted delivery of active agents to specific sites of action.

The composition of the invention comprises diazepam covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a
20 heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and
25 turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

5 The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex,
10 PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized
15 adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions,
20 sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is
25 particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-diazepam conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

5 **Acid/N-terminus conjugation**

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product
10 precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole
15 followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene
20 produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified
25 using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated

solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

5 **Preparation of γ -Alkyl Glutamate**

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered,
10 dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for
15 several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes
20 homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically
25 overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 diazepam covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein diazepam is covalently attached to a side
chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein diazepam is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing diazepam from said composition in a pH-dependent manner.

15 19. A method for protecting diazepam from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of diazepam from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching diazepam to said polypeptide.

20 21. A method for delivering diazepam to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
diazepam covalently attached to said polypeptide.

25 22. The method of claim 21 wherein diazepam is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein diazepam is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

A composition comprising a polypeptide and diazepam covalently attached to the polypeptide. Also provided is a method for delivery of diazepam to a patient comprising administering to the patient a composition comprising a polypeptide and diazepam covalently attached to the polypeptide. Also provided is a method for protecting
15 diazepam from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of diazepam from a composition comprising covalently attaching it to the polypeptide.

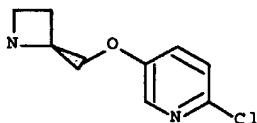
A NOVEL PHARMACEUTICAL COMPOUND AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to cholinergic channel modulator, as well as methods for protecting and administering cholinergic channel modulator. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and
10 occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Cholinergic channel modulator is a known pharmaceutical agent that is used in
15 the treatment of pain. Its chemical name is (R)-2-chloro-5-(2-azetidinylmethoxy)pyridine. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability
20 of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

25 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf
5 life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as
10 resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the
15 stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example,
20 copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties
25 to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

5 The present invention provides covalent attachment of the active agent (cholinergic channel modulator) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching cholinergic channel modulator to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier
10 peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the
15 carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising cholinergic channel modulator microencapsulated by a polypeptide.

20 The invention provides a composition comprising a polypeptide and cholinergic channel modulator covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and
25 one or more synthetic amino acids.

Cholinergic channel modulator preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the

polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol
5 and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant
10 preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestible tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the
15 composition in a pH-dependent manner.

The invention also provides a method for protecting cholinergic channel modulator from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering cholinergic channel modulator to a patient, the patient being a human or a non-human animal, comprising
20 administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, cholinergic channel modulator is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, cholinergic channel modulator is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another
25 preferred embodiment, the composition further comprises a microencapsulating agent and cholinergic channel modulator is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, cholinergic channel modulator is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, cholinergic channel modulator is released

from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic
5 release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

- (a) attaching cholinergic channel modulator to a side chain of an amino acid to form an
10 active agent/amino acid complex;
- (b) forming an active agent/amino acid complex N-carboxyanhydride (NCA)
from the active agent/amino acid complex; and
- (c) polymerizing the active agent/amino acid complex N-carboxyanhydride
(NCA).

15 In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, cholinergic channel modulator and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide
20 and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an
25 ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the
30 following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

5 The present invention provides several benefits for active agent delivery. First, the invention can stabilize cholinergic channel modulator and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of cholinergic channel modulator. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be
10 enhanced. The invention also allows targeted delivery of active agents to specific sites of action.

Cholinergic channel modulator is the subject of WO 96/40682 (1996), based on priority US application 474873, herein incorporated by reference, which describes how to make that drug.

15 The composition of the invention comprises cholinergic channel modulator covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a
20 heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and
25 turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's
5 decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other
10 hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

15 Other factors such as π - π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

20 Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have
25 profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond
 5 between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and
 10 drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be
 15 controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

<u>Amino acid</u>	<u>MW</u>	<u>Active agent</u>	<u>MW</u>
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B ₆ (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B ₂ (Riboflavin)	376
		Vitamin D ₂	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the
 20 stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is

5 polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action

10 for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is

15 distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or

20 the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazines to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

25 In the present invention, cholinergic channel modulator is covalently attached to the polypeptide via the amine group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-cholinergic channel modulator conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids
5 and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be
10 stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C.
15 The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

20 In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product
25 is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ -Alkyl Glutamate

There have been over 30 different γ -alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ -alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ -Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ -alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ -Alkyl Glutamate-NCA

γ -Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ -Alkyl Glutamate]

γ -Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically

overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,
5 various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
5 cholinergic channel modulator covalently attached to said polypeptide.
2. The composition of claim 1 wherein said polypeptide is an oligopeptide.
3. The composition of claim 1 wherein said polypeptide is a homopolymer of a
naturally occurring amino acid.
4. The composition of claim 1 wherein said polypeptide is a heteropolymer of
10 two or more naturally occurring amino acids.
5. The composition of claim 1 wherein said polypeptide is a homopolymer of a
synthetic amino acid.
6. The composition of claim 1 wherein said polypeptide is a heteropolymer of
two or more synthetic amino acids.
- 15 7. The composition of claim 1 wherein said polypeptide is a heteropolymer of
one or more naturally occurring amino acids and one or more synthetic amino acids.
8. The composition of claim 1 wherein cholinergic channel modulator is
covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.
9. The composition of claim 1 further comprising a microencapsulating agent.
- 20 10. The composition of claim 9 wherein said microencapsulating agent is
selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar
and a salt.
11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

5 14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

10 16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein cholinergic channel modulator is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing cholinergic channel modulator from said composition in a pH-dependent manner.

15 19. A method for protecting cholinergic channel modulator from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of cholinergic channel modulator from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching cholinergic channel modulator to said polypeptide.

20 21. A method for delivering cholinergic channel modulator to a patient comprising administering to said patient a composition comprising:
a polypeptide; and
cholinergic channel modulator covalently attached to said polypeptide.

25 22. The method of claim 21 wherein cholinergic channel modulator is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein cholinergic channel modulator is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

5 25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

10 Abstract

A composition comprising a polypeptide and cholinergic channel modulator covalently attached to the polypeptide. Also provided is a method for delivery of cholinergic channel modulator to a patient comprising administering to the patient a composition comprising a polypeptide and cholinergic channel modulator covalently
15 attached to the polypeptide. Also provided is a method for protecting cholinergic channel modulator from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of cholinergic channel modulator from a composition comprising covalently attaching it to the polypeptide.

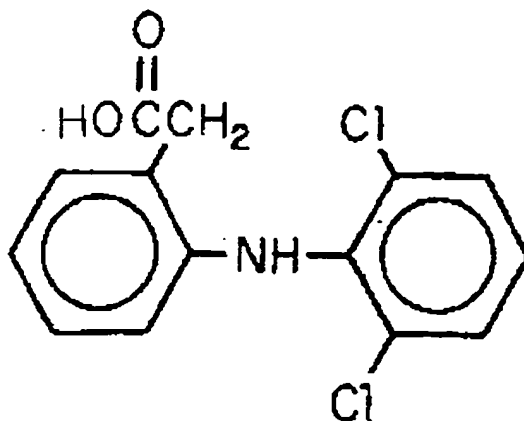
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING DICLOFENAC AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

5 The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to diclofenac, as well as methods for protecting and administering diclofenac. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of
10 the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

 Diclofenac is a known pharmaceutical agent that is used in the treatment of acute
15 and chronic rheumatoid arthritis. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its chemical name is potassium (o-(2,6-dichloroanilino)-phenyl)acetate. Its structure is:



 The novel pharmaceutical compound of the present invention is useful in
20 accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered

product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

5 Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another
10 invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of
15 cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme
20 degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example,
25 copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.